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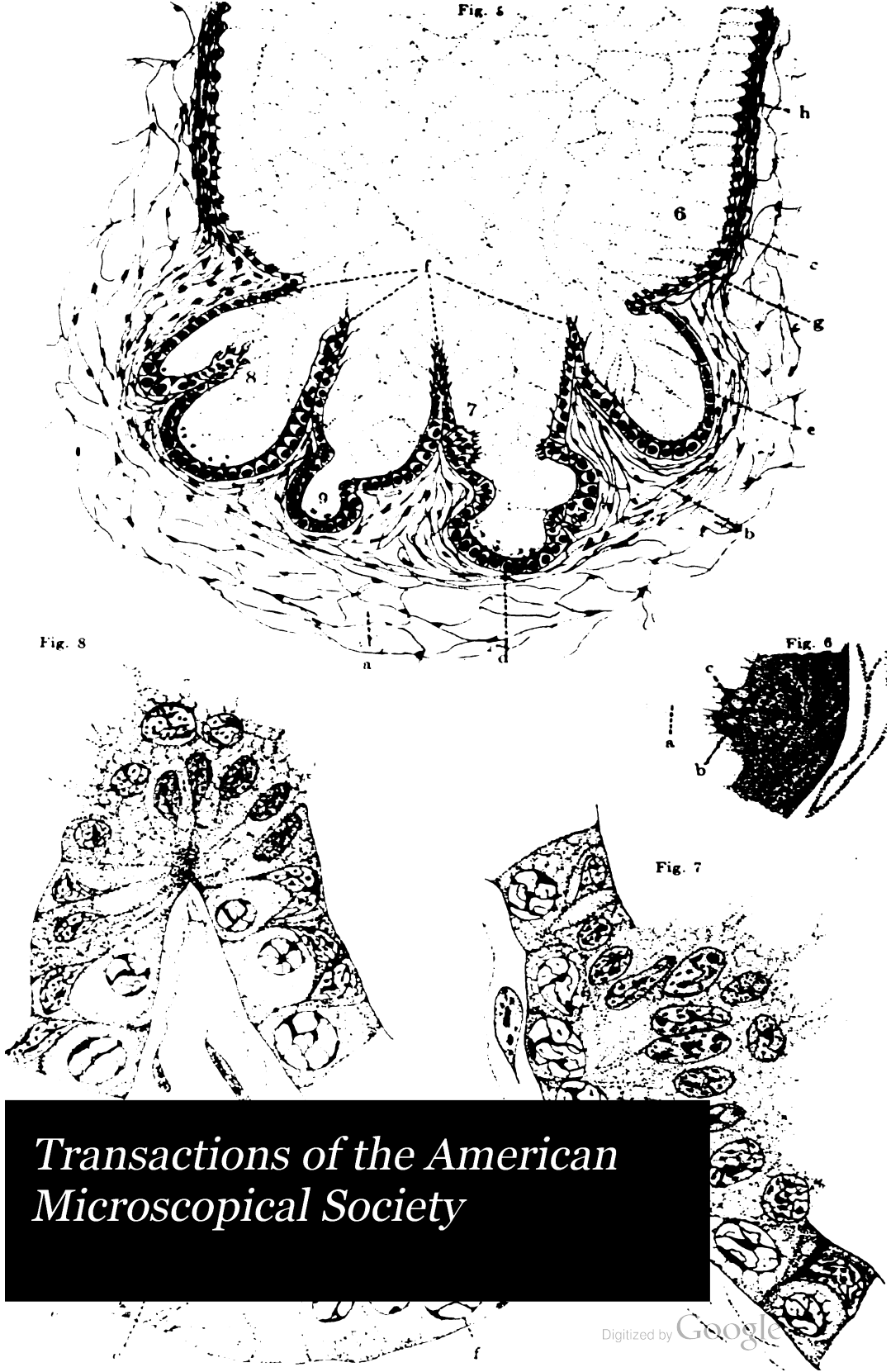

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JANUARY
1915

VOLUME XXXIV No. 1

George E. Coghill,

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T. W. GALLOWAY,
Secretary.

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TRANSACTIONS
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Vol. XXXIV JANUARY, 1915 No. 1

ADDRESS OF THE PRESIDENT FOR 1914.

Some Points in the Development of the Nose.

By Charles Brookover.
Medical Department of the University of Arkansas.

No exhaustive treatment of the subject is here attempted. For the most part this article is a review of the literature and attempts to set certain recently discovered facts into relation with older observations on the nose and its development. Whatever may be offered in the way of theoretical conclusions we trust will be of value in pointing the way to a more clear understanding of our problems yet unsolved.

Although Max Schultze showed in 1862 that the adult olfactory nerve arises from cells located in the nasal epithelium many anatomists and embryologists of later date persisted in making it homologous with a typical ganglionated sensory nerve of the body region. Some anatomists looked upon the olfactory bulb as the ganglion. With the advent of the Golgi method and the description of the relation of the mitral cells and their glomeruli to the olfactory tract this idea had to be abandoned. Moreover it was shown embryologically that the olfactory bulbs, so often quite far removed from the brain as in the sharks, develop from the forebrain and are not in any way similar to dorsal root ganglia.

Among those who found a ganglion in the developing olfactory nerve may be mentioned such men as Milnes Marshall, Balfour, von Kölliker, Beard and Chiargui. The idea in the minds of most of these men was that the head nerves are segmental and homologous

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with those of the body. These attempts to homologize the cranial with the spinal nerves may be attributed to the trend of zoological thought in the time in which they wrote. The recently accepted evolution theory of Darwin led embryologists to look for evidences of the derivation of the vertebrates from segmented forms like the annelids. On this assumption the cranial nerves, even the olfactory, might be found to have a ganglion in its development. That these men found a ganglion on the olfactory nerve can no longer be doubted.

In all chordates higher than Amphioxus the first evidence of the olfactory organ is a thickening of the epithelium of the embryo not far from the closure of the neuropore. Following Kupffer we may designate this the nasal placode. Kupffer found the earliest placode to be single in the Cyclostomes and argued for a primitive monorhinal condition in vertebrates. I think we may agree with Karl Peter that the monorhinal condition that seems to exist in larval Cyclostomes is secondary, on account of their parasitic habits, perhaps. At any rate the olfactory nerves are paired and there are two sets of folds in the Schneiderian membrane of adult Cyclostomes. In other higher vertebrates these placodes are always paired and according to Peter, distinct from each other from the first. He was unable to confirm the finding of an unpaired connection between the two as cited by Gage for a human embryo of 4.3 mm. length. I thought I found an unpaired placode in Amia, but the matter needs further investigation to learn whether this unpaired placode is not a cord of cells left from the closure of the neuropore.

In pig and man the first thickening of the ectoderm occurs before the embryo attains a length of 5 mm., and later the depression that deepens into the nasal sac is soon evident. At this time there is considerable thickness of loose connective tissue mesoderm intervening between the nasal placodes and the neural tube. This makes it easy to determine that the olfactory nerve grows inward from the placode and does not originate from the neural tube. In lower forms like some of the fishes, the olfactory placode is in contact with the neural tube when it can first be detected with certainty.

The veteran embryologist, Wm. His, Jr., working on mammalian embryos, was the first to cause the idea that the olfactory nerve

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is derived from the ectoderm exclusively, to pass current in the realm of embryology. Also, he found a ganglion, wandering out from the nasal epithelium to produce neuroblasts for the formation of the fila olfactoria as well as the formation of supporting elements. He believed that the true nervous elements of the ganglion pass over to the olfactory bulbs. This would leave an adult olfactory nerve free of ganglion cells in accordance with the adult condition described by Schultze cited above. This origin of the ganglion from the ectoderm through the thickening of the nasal epithelium is in direct contrast with the ideas of Beard and others who sought in the ganglion a homologue of neural crest. Disse is the other noted investigator of the development of the olfactory nerve whose work seems generally accepted by embryological writers today. Working on birds, he confirmed the work of His in finding the ganglion to arise from the olfactory epithelium. He showed that there was never a ganglion of considerable size. By the Golgi process he discovered that there were only a very few true nerve cells. In older embryos these nerve cells were found nearer and nearer to the olfactory bulbs. The majority of the cells migrating from the nasal epithelium were believed to become sheath cells of the fila olfactoria.

Bedford followed up the work of Disse on the birds by similar methods both cytological and neurological applied to the pig. He confirms the findings of His and Disse as to the origin of cells along the fila olfactoria from the nasal epithelium. He says the majority of these cells do not form nerve cells. He mentions the suggestion of Disse that they form supporting cells, but closes by saying he did not determine their fate. In a series of papers undertaken to clear up the embryological history of the nervus terminalis, I have come to the same conclusion as the authors cited above, viz., that a ganglion arises in the course of the olfactory nerve whose cells come from the nasal epithelium. No evidence of origin of any part of this ganglion from the brain or any other source than that named, is at hand.

It is the presence of the nervus terminalis in all groups of vertebrates from sharks to man as shown by a series of papers published during the past decade by various authors that calls for a new examination of the relation of the embryological ganglion on, or near,

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the olfactory nerve. The older observations of the ganglion on the olfactory nerve did not take account of the nervus terminalis. The embryological observations undertaken since the discovery of the nervus terminalis have not been in complete accord in regard to the relation of the embryological ganglion to the olfactory nerve and the nervus terminalis. Locy, the discoverer of the nervus terminalis in the sharks, was at first of the opinion that the nervus terminalis has a common origin with the olfactory nerve. Later he came to the view that there is a separate origin for the two and

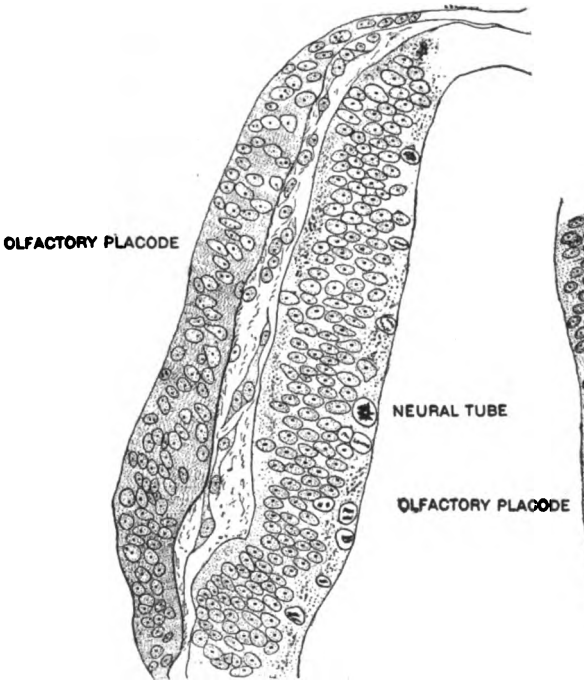

FIG. 1

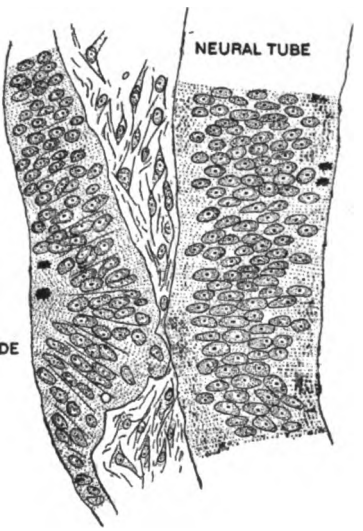

FIG. 2

FIG. 1. Section of a transverse series through the olfactory placode of *Chrysemys martinata* nearly 4 mm. long. On account of head flexure the section is approximately horizontal in this region, as are all of the others. X 250.

FIG. 2. Similar section of 4 mm. Chrysemys embryo, showing slight advance in development of olfactory placode which is now in contact with the neural tube. X 250.

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that the nervus terminalis is a separate segmental nerve of a more anterior segment than the olfactory.

Later Belogolowy working on selachians, found both the olfactory nerve and the nervus terminalis to be derived from what he designates a primitive olfactory ganglion arising out of the olfactory thickening of the ectoderm. My observations on series of graded embryos of fresh-water fishes as previously published, are in accord with Belogolowy except that I do not see the need or propriety of calling the ganglion a primitive olfactory ganglion. No evidence has presented itself to me that fila olfactoria are developed from the ganglion. The evidence is in favor of the origin of olfactory neuraxones from cells that never wander from the Schneiderian membrane of the nasal mucosa.

The following account of the development of the olfactory nerve and the nervus terminalis in relation to the ganglion in the turtle, Chrysemys marginata, will serve to illustrate the embryological history of the two nerves. The history is the same as that found in the fishes. I am indebted to my former pupil, Mr. Albert B. Myers, for the drawings and help in preparation of the sections.

When the Chrysemys embryo measures 4 mm. total length the ectodermal thickening of the nasal placode can be recognized over a wide area in front of the optic vesicles. The nuclei of this epithelium occupy three or four indefinite layers (fig. 1). The thickness is slightly greater in the posterior part of the placode. The section is cut approximately horizontal on account of the head flexure. At the anterior end of the neural tube the ectoderm is in contact with the neural tube, but over the greater part of the extent of the nasal placode scattered mesodermal cells are interposed. The mesodermal cells increase in number and compactness farther ventrally and a small capillary could be detected.

Figure 2 is of a size marked the same as the specimen from which the preceding figure was drawn but shows a slight advance over it. The placode has thickened and come into contact with the neural tube. Adjacent section shows limiting membranes broken down and a slender protoplasmic bridge extending from the placode into the neural tube. The mesodermal elements are more compacted than in the previous stage.

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When the embryo has reached a slightly larger size (fig. 3) the nasal epithelium has increased in thickness and number of cells. The basement membrane is wanting over much of its deeper surface and a strand of fibers pierces the neural tube. Adjacent to the strand of fibers can be seen a clump of cells just emerging from the nasal placode. They are slightly larger than the remainder of the placodal cells and show an irregular disturbed arrangement, whereas the placodal cells are disposed in rows perpendicular to

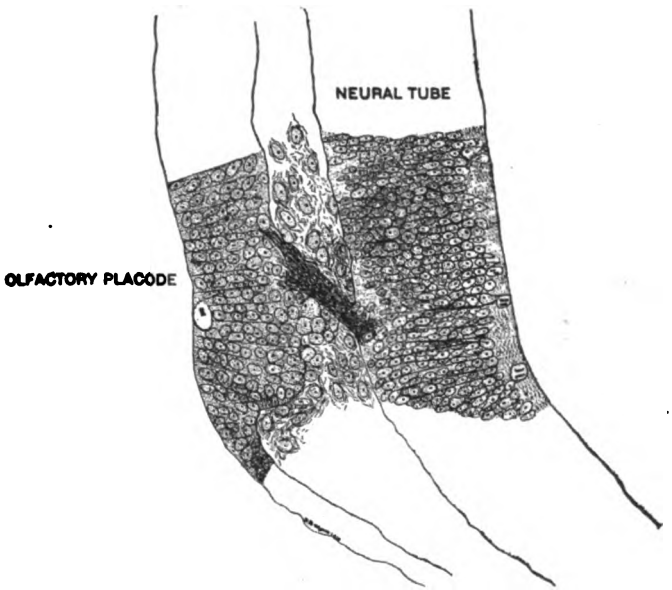

FIG. 3

FIG. 3. Section of Chrysemys about four and one-half mm. long. The olfactory nerve fibers are beginning to develop and the cells of the nervus terminalis are migrating from the nasal placode. X 250.

the outer surface where the mitotic figures are always plentiful. It is from these emergent cells that the ganglion on the olfactory nerve arises.

In an embryo of the same length as the above, which shows by the nasal pits a degree of advance in development, the olfactory

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nerve is shown cut through its entire length (fig. 4). The cells are seen to occupy a position in the nerve and on its lateral border. The cells here shown nearer to the brain tube than the olfactory epithelium, are not the only ones along the nerve, as is evident from other sections.

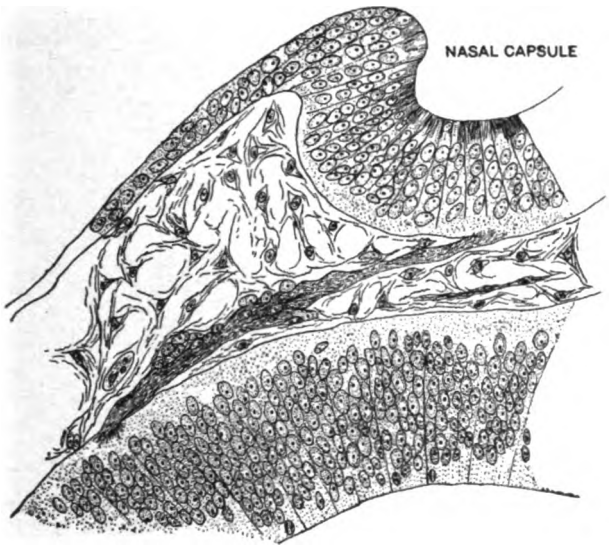

FIG. 4

FIG. 4. Chrysemys 5 mm. long with nasal pit forming and a number of cells of the olfactory ganglion intimately associated with the olfactory nerve. X 250.

When the embryo has increased to nearly six mm. length, the size of the olfactory nerve is larger and the number of cells along it is more numerous (fig. 5). The artist has drawn all the cells to appear as neuroblasts with vesicular nuclei. Whether all are of this character is uncertain. The main ganglion is located near the nasal epithelium where the olfactory nerve breaks up into smaller twigs.

We have not followed the development of the ganglion in the turtle through to the nervus terminalis of the adult as we did in the fishes, but we have no doubt that such an adult structure exists. Johnston has described the nervus terminalis in another turtle,

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practically adult. We have followed out the early developmental history in a cyprinoid fish (probably a horned Dace, work still unpublished) and found the evidence the same, viz., that the cells

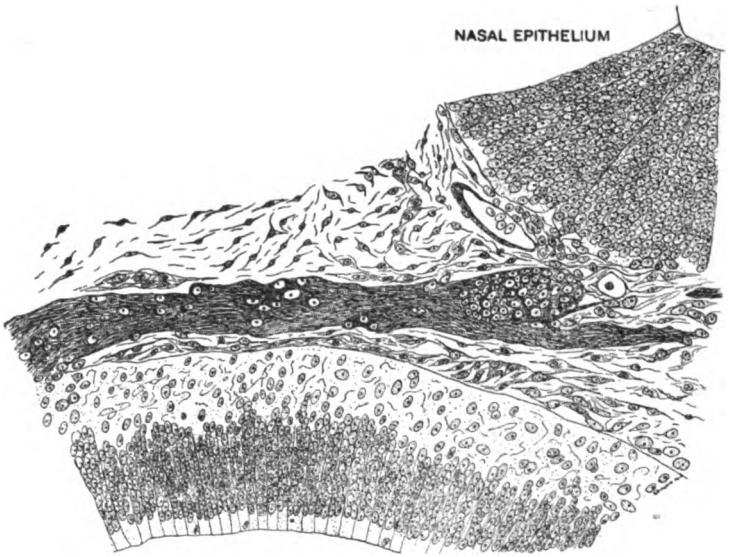

FIG. 5

FIG. 5. Chrysemys of nearly 6 mm. length showing the ganglion of the nervus terminalis near the blood vessels at the base of the nasal epithelium. These cells show the characters of neuroblasts. There are other cells scattered along the olfactory nerve as far as its union with the brain. X 250.

along the olfactory nerve arise from the nasal epithelium and that these cells give rise to the nervus terminalis. That some of these cells may give rise to the sheath cells of the olfactory nerve is very probable. As the sheath cells are distributed as far posteriorly as the olfactory bulbs and there is often a ganglion terminale not far from the olfactory bulbs, we readily harmonize our findings with the statements of His and Disse, noted above, that cells from the embryological ganglion pass over to the olfactory bulbs.

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DISCUSSION

If other embryological investigations show that the above account of the origin of the nervus terminalis from the olfactory placode is correct, it would seem to me to prove that the nervus terminalis is a component of the olfactory nerve. I have previously taken this view of the matter and Johnston has emphasized it. That the nervus terminalis is not homologous with the nerve to Jacobson's organ is quite clear. The nervus terminalis is present in forms where the vomeronasal organ is absent or rudimentary as in man. Read has shown that Jacobson's organ has nerve fibers of the true osmatic type. Although the nervus terminalis is frequently intimately associated with the ramus to the organon vomeronasale it may exist side by side with the nerve to Jacobson's organ, as Johnston has shown, or be distributed in part to a region anterior to the organ of Jacobson as demonstrated by Huber and Guild. We are in no position to state definitely the relation existing between the organ of Jacobson and the nervus terminalis so long as we are uncertain of the function of either of them. With regard to Jacobson's organ we may incline to the view expressed by Peter that it serves various functions in the different vertebrate classes. It may be glandular in part and it may serve for sex recognition, for testing the expired air, or for recognition of harmful food-stuff in the mouth. If it serves these various functions it may very well be that the structure is not strictly a homologous organ in all the vertebrates where found. Evidently it is not so essential as the nervus terminalis which seems everywhere present in the vertebrates so far as known.

I have been hunting for a type of vertebrate where the nervus terminalis is hypertrophied so as to gain further evidence of its function. No such case has been found. The nerve is developed *pari passu* with the development of the nasal sac. This is in keeping with the function that I have suggested previously, that it is in part an autonomic nerve. The opinion of Huber and Guild is in accord with this view with the reservation that its central connection does not conform to their idea of a typical autonomic nerve. It may be that it contains general somatic sensory fibers as suggested by Johnston. The recent work of McKibben on the sharks by intra vitam

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methylene blue emphasizes two facts. The cells are not constant in position or grouping and they are not of a type such as we should expect in a typical somatic sensory ganglion. Instead of being T-shaped unipolar cells, or bipolar, they are largely of multipolar variety so far as impregnated in his various preparations. Huber and Guild noted many multipolar cells in the rabbit and they have been conspicuous in the fishes that have come under my observation.

In addition to the common embryological origin of the nervus terminalis and the olfactory nerve noted above is to be mentioned the observation of Pinkus, the discoverer of the nervus terminalis in fishes, that it has the same peripheral distribution (nasal mucous membrane), the same central ending (prosencephalon), and the same nature of supporting (sheath) cells without medullation, as has the olfactory nerve. In general these statements hold true today in light of our extended knowledge. However, there seems to be a central connection with parts of the brain more posterior than the osmatic region of the forebrain. McKibben traced the central path to the interpeduncular region of the mesencephalon, and in Amia there is apparently a tract reaching as far as the optic chiasm or farther.

The exclusively placodal origin of the nervus terminalis and the olfactory nerve does not bring the nasal segment into closer correspondence with the other cranial nerves. The homologue of the neural crest which the early workers on the cranial nerves sought does not seem to be present. However, it does seem to emphasize the close relationship between the sense of smell and that of taste. It is quite well known that there is a marked physiological correspondence in the fact that we discriminate between taste and smell with difficulty in many instances. Herrick has discussed their probable origin from a common chemical sense, the olfactory organ into an exteroceptor and the taste-buds into interoceptors. Landacre has shown that epibranchial placodes occur on those nerves only that contain gustatory fibers. Thus it might seem that the sense of smell and the sense of taste originated from cells that were located not in the main axial group included in the neural plate, but from cells scattered in the skin as placodes, primitive sense organs.

When we attempt the comparison of the sense of smell and the sense of taste on the morphological side we meet with some

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difficulties. It is true there are arguments that can be adduced to show that the nasal cavities are probably gill slits. The nose is quite commonly intimately connected with the mouth, which is admittedly a gill slit. I have gone so far as to figure a supply of blood to the nose of Amia through the internal and external carotid arteries similar to that given to the pseudobranch. The nasal folds in the Schneiderian membrane of fishes can be compared to gill filaments and Blaue went so far as to compare the groups of osmatic cells in some fishes to taste-buds. I have noticed an apparently similar grouping in the nasal cavity of young Amblystomæ. But if the structures in the nose sometimes resemble taste-buds, they are not really similar. Gustatory cells are conceded to have very brief basal processes not reaching beyond the taste-bud, while olfactory cells send their axones into the glomeruli of the olfactory bulb and leave the olfactory nerve without a ganglion except the nervus terminalis. This is not thought to be large enough to serve the transmission of the sense of smell if it made the proper cranial connections. Thus, the osmatic sense retains that primitive position of its neurones in the integument like invertebrates, as was first pointed out by Retzius, I believe. It may be that an early development of a protective nasal capsule has lessened the necessity for withdrawal of the neurones from the outside. It has occurred to me that the residence of the neurone body in the epithelium where the chemical stimuli are received may add to the delicacy of smell over taste as shown by Parker and Stabler in experiments on each other with dilutions of alcohol.

We may summarize the development of the olfactory nerve by saying that the present evidence points to the embryological as well as morphological distinctness from other nerves. It is more closely allied in its development to taste-bud components of cranial nerves, but its neurones remain in the ectoderm. The development of the nervus terminalis along with the olfactory nerve from the same placode is unique among nerves that are somatic sensory or sympathetic.

Little Rock, Arkansas.
February 22, 1915.

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SOME FRESH-WATER NEMATODES OF THE DOUGLAS LAKE REGION OF MICHIGAN, U. S. A.*

MARGARET V. COBB. (Descriptions by N. A. COBB).

I. Introduction.

This account of the fresh-water nematodes of the Douglas Lake region is based on work made possible by a research fellowship from the University of Michigan for work at the Biological Station at Douglas Lake. The work was carried on in the summer of 1913, under the supervision of Professor Frank Smith of the University of Illinois. Most of the specific determinations have been made by Dr. N. A. Cobb, Department of Agriculture, Washington, D. C., and all have received his confirmation. I wish to acknowledge also the hospitality of the Bureau of Plant Industry, of the Department of Agriculture, during a part of the work.

II. The Region.

Douglas Lake liçs in the sandy country of the northern end of the lower peninsula of Michigan, about halfway between Petosky and Mackinaw. It is about four miles long by one to two miles wide, and is one of many small lakes in the region. Collections were made from the waters of the lake itself; from its beach-pools of various ages; from its inlet, Bessey Creek; its outlet, Maple River; from a sphagnum bog cut off from the lake; and from three neighboring lakes. Of these, Monroe Lake lies about a mile north of Douglas Lake. Lancaster Lake, also to the north, is connected with Douglas Lake by Bessey Creek, which flows from the former into the latter. Dead Lake, close to Douglas Lake, has dwindled to a mere series of marshy pools. Collections were made in the following situations:

1. Maple River, entrance. Sand and mud near bank. About eighty-five specimens.

2. Maple River, short distance from entrance. Sand and mud near bank. About eighteen specimens.

3. Fairy Island, south shore. Detritus on sand, among rushes. Eight specimens.

* Publication No. 30 from the Biological Station of the University of Michigan.

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4. Beach pool, south end of North Fish Tail Bay. Sand and mud, near crayfish burrows. Eight hundred specimens.

5. Large beach pool, Pine Point. Among moss. Two hundred fifty specimens.

6. Large beach pool, Pine Point, east end. Sand and mud. Thirty-three specimens.

7. Old beach pool, Pine Point. Mud, among rushes. One hundred specimens.

8. New beach pool, Pine Point. Sand and mud. One hundred seventy-five specimens.

9. Bessey Creek, middle bridge. Sand and mud near bank. Very small collection. About one hundred specimens.

10. Lancaster Lake, north shore. Two specimens.

11. Inlet of Lancaster Lake. Four specimens.

12. Monroe Lake. Among smartweed growing in water along shore. Eighteen specimens.

13. Dead Lake. Pool near roadside bridge, among moss and algæ. One hundred fifty specimens.

14. Bryant's Bog, east end. Among sphagnum. Thirteen hundred specimens.

15. Bryant's Bog, west end. Among sphagnum. Six hundred specimens.

III. Preparation of Material.

Free-living nematodes are abundant in this region, as elsewhere. They were collected by taking samples of the sand or mud and water of the pool or stream bottom, and of the aquatic vegetation. These were washed through a series of graded sieves, from coarse to fine, which removed the coarser debris, until examination with a lens showed that nematodes also were caught on the sieve. The collection was then allowed to settle for five minutes or more, and the superfluous water poured off. The nematodes were killed and fixed by adding to this watery mud an equal quantity of boiling hot saturated solution of corrosive sublimate.

Staining and mounting these small specimens is a less summary matter. Each sample was treated as follows. The sediment was examined, a little at a time, in a Syracuse watch-glass under a dissecting lens; the nematodes were picked out one at a time with a bamboo

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splinter, and placed in water in the object-box of a differentiator[1], in which they were gradually passed up through the grades of alcohol to 80% alcohol. At this point they were treated with acid alcohol to dissolve out impurities (10 drops conc. HCl to 100 cc. 80% alc.) and overstained with Mayer's acid carmine according to the following formula:

Carmine..... 4 grams
H_2O..... 15 cc.
HCl..... 30 drops
Add 95 cc. of 85% alcohol, boil until carmine is dissolved, neutralize with ammonia until carmine begins to precipitate, filter through glass wool.

For differentiation of the tissues acid alcohol was used (4 drops conc. HCl to 100 cc. 80% alc.). The specimens, still in the object-box, were then passed up to and through absolute alcohol and turpentine to thin balsam. This was done without removing the object-box from the differentiator, except to remove it to another type of differentiator when the change to heavier fluids began. The object-box was now opened in thin balsam in a Syracuse watch-glass, and the nematodes mounted in balsam.[2] From ten to one hundred, according to size, can be arranged in one drop of balsam without much crossing of specimens. This also is best done under magnification; it is convenient to have two dissecting microscopes, keeping the watch-glass of specimens under one, and the slide which is being prepared under the other. These methods were developed by Dr. N. A. Cobb.

IV. Results.

These collections from the Douglas Lake region have yielded thirty-six species, two-thirds of which are new species. They belong to twenty-seven genera; two, *Dolichodorus* and *Tylencholaimellus*, are new, and eight others have not previously been

[1]For description, see New South Wales Dep't Agriculture Miscellaneous Publication No. 215. "Nematode Parasites; Their Relation to Man and Domesticated Animals". (N. A. Cobb.)

[2]The mounts were made on extra thin coverglasses held in perforated aluminum frame of regulation size, and covered by a smaller thin coverglass. The specimens may then be examined from either side under short focus oil immersion lens. This procedure is necessary for determining the finer histological details. Aluminum slides may be obtained from F. Cobb, Falls Church, Virginia.

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recorded from North America. There is little doubt that further collections will add to this list; for while certain ubiquitous species were common to most of the collections, and many were taken from more than one situation, each of the larger collections yielded some forms found in none of the others. For some time to come each new collection may be expected to yield new forms.

The present species are as follows:

A. Known species (see bibliography for descriptions)
   1. Aphelenchus microlaimus Cobb. (1).
   2. Diplogaster fictor, Bastian (3).
   3. Achromadora minina, Cobb (1).
   4. Cryptonchus nudus, Cobb (2).
   5. Plectus tenuis, Bastian (4).
   6. Chronogaster gracilis, Cobb (2).
   7. Mononchus longicaudatus, Cobb (1).
   8. Diploscapter coronata, Cobb (2, 1).
   9. Monhystera filiformis, Bastian (4).
   10. Tripyla affinis, de Man (4).
   11. Trilobus longus (Leidy) Bastian (3).
   12. Anonchus monhystera, Cobb (2).

B. New species, described in Trans. American Micr. Soc., for April, 1914 (3).
   1. Dolichodorus n. g. heterocephalus.
   2. Microlaimus fluviatilis.
   3. Rhabdolaimus minor.
   4. Cephalobus sub-elongatus.
   5. Teratocephalus cornutus.
   6. Prismatolaimus stenurus.

C. New species here described.
   1. Tylencholaimellus n. g. diplodorus n. sp.
   2. Actinolaimus propinquus, n. sp.
   3. Dorylaimus sulcatus, n. sp.
   4. Ironus minor, n. sp.
   5. Mononchus lacustris, n. sp.
   6. Cyatholaimus fluviatilis, n. sp.
   7. Chromadora inornata, n. sp.
   8. Ethmolaimus gracilicaudatus n. sp.
   9. Aphanolaimus viviparus, n. sp.
   10. Aphanolaimus communis, n. sp.
   11. Prismatolaimus digitatus, n. sp.

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D. Undetermined species.

1. Dorylaimus sp.
2. Dorylaimus sp.
3. Dorylaimus sp.
4. Dorylaimus sp.
5. Plectus sp.
6. Rhabditis sp.
7. Monhystera sp.

TABLE SHOWING LOCAL DISTRIBUTION OF THE SPECIES

	Monroe Lake	Lancaster Lake	Inlet to Lancaster Lake	Fairy Island	Maple River	Bessey Creek	New Beach pool	Beach pool North Fish Tail Bay	Beach pool Pine Pt. sand and mud	Old Beach pool rushes	Beach pool Pine Pt. moss	Dead Lake	Bryant's Bog
Aphelenchus microlaimus	..	..	..	..	..	..	..	×	..	..	..	..	..
Tylencholaimellus diplodorus	..	..	..	..	×	..	..	..	..	..	..	..	..
Dolichodorus heterocephalus	..	..	..	..	..	..	..	..	..	..	×	..	..
Dorylaimus sulcatus	..	..	×	..	×	×	..	×	..	..	×	..	..
Dorylaimus spp.	×	..	..	×	×	×	..	×	×	×	×	×	×
Actinolaimus propinquus	..	..	..	..	×	×?	..	×	×	..	..	..	..
Ironus minor	..	..	..	..	..	×	..	×	×	..	×	..	×
Mononchus longicaudatus	..	..	..	..	..	..	..	..	..	..	×	..	..
Mononchus lacustris	..	..	..	..	..	×	..	×	×	..	×	..	×
Diplogaster fictor	×	..	..	..	..	..	..	..	..	..	..	×	×
Cyatholaimus fluviatilis	..	..	..	..	×	..	..	..	..	..	..	..	..
Achromadora minima	..	..	..	..	..	..	..	×	..	..	..	..	..
Chromadora inornata	..	..	..	..	×	×	..	..	..	..	..	..	..
Ethmolaimus gracilicaudatus	..	..	..	..	×	..	..	..	..	..	..	..	×?
Microlaimus fluviatilis	..	..	..	×	×	×	×	×	×	×	..	..	×
Cryptonchus nudus	..	..	..	..	×	×	..	×	×	×	×	..	..
Plectus tenuis	..	..	..	..	×	×	..	×	×	×	×	..	×
Plectus sp.	..	..	..	..	..	..	..	..	..	..	..	×	..
Chronogaster gracilis	..	×	..	..	..	×	×	×	×	×	×	..	×

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	Munroe Lake	Lancaster Lake	Inlet to Lancaster Lake	Fairy Island	Maple River	Bessey Creek	New Beach pool	Beach pool North Fish Tail Bay	Beach pool Pine Pt. sand and mud	Old Beach pool rushes	Beach pool Pine Pt. moss	Dead Lake	Bryant's Bog
Rhabditis sp.	..	..	..	..	..	..	×	..	..	..	..	..	..
Rhabdolaimus minor	..	..	..	..	..	×	×	×	×	×	×	..	×
Cephalobus sub-elongatus	..	..	..	..	..	..	..	..	..	..	..	..	×
Teratocephalus cornutus	..	..	..	..	×	..	..	×	×	..	×	..	×
Aphanolaimus viviparus	×?	..	×	..	×	×	×	×	×	×	×	..	..
Aphanolaimus communis	×?	..	..	×	×	×	..	×?	×	..	×	..	..
Prismatolaimus stenurus	..	..	..	..	..	..	..	×?	×	..	..	×	×
Prismatolaimus digitatus	..	..	..	..	..	..	..	×?	×	×	..	×	×
Monhystera filiformis	×?	..	..	..	×	×?	×	×	×	×	×	..	×
Monhystera sp.	×?	..	..	..	..	×?	..	×	..	..	×	..	..
Trilobus longus	×	×	..	×	×	×	×	×	×	..	×	..	..
Tripyla affinis	..	..	..	..	..	×	..	×	..	..	×	..	..
Anonchus monhystera	..	..	..	×	×	×?	×	..	..	..	..	..	..
Diploscapter coronata	..	..	..	..	..	..	..	..	..	..	..	..	×

V. Key to the Genera Found in These Collections.

1. (10) Oral end armed with protrusile spear or sting..... 2
2. (5) Spear with bulbous base..... 3
3. (4) Œsophagus with median bulb only.....Aphelenchus, Bastian
4. (3) Œsophagus with median bulb only.....Tylencholaimellus, Cobb
5. (2) Spear without a bulbous base..... 6
6. (7) Œsophagus with median bulb; males with bursa..... ..... Dolichodorus, Cobb
7. (6) Œsophagus with only an elongated posterior swelling..... 8
8. (9) Pharynx simple, male supplementary organs not in fascicles ..... Dorylaimus, Dujardin
9. (8) Pharynx with complicated radiate framework, male supplementary organs in fascicles.....Actinolaimus, Cobb

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10. (1) Oral end without protrusile spear or sting..... 11
11. (28) Pharynx armed with one or more refractive chitinous teeth 12
12. (13) Number of teeth three; equal, small, mobile, well forward near the mouth .....Ironus, Bastian
13. (12) Number of teeth one; or more and unequal..... 14
14. (15) Teeth, especially dorsal tooth, usually massive; thick, more or less prominently papillate lips closing over the pharynx .....Mononchus, Bastian
15. (14) Teeth small, often only one, then dorsal; lips with inconspicuous papillæ; pharynx moderate..... 16
16. (27) Œsophagus usually with one or two bulbs..... 17
17. (18) Bulbs two, spinneret absent.....Diplogaster, M. Schultze
18. (17) Bulb one, then cardiac; or none. Spinneret present..... 19
19. (20) Pharynx cupshaped, then conoid, longitudinally ribbed; without œsophageal bulb.....Cyatholaimus Bastian
20. (19) Pharynx less conspicuously ribbed; œsophagus with a distinct cardiac bulb..... 21
21. (26) Dorsal tooth well developed..... 22
22. (25) Pharynx cyathiform, then conoid, joining the œsophagus rather indefinitely 23
23. (24) Dorsal tooth opposed by one or more small teeth, amphids spiral, at side of head.....Achromadora, Cobb
24. (23) Dorsal tooth single, amphids invisible or inconspicuous, towards front of head.....Chromadora, Bastian
25. (22) Pharynx cyathiform, then prismoid, ending behind very definitely; amphids distinct.....Ethmolaimus, De Man
26. (21) Dorsal tooth very small, circular amphids well developed... .....Microlaimus, De Man
27. (16) Œsophagus plain .....Cryptonchus, Cobb
28. (11) Pharynx without teeth..... 29
29. (42) Œsophagus with bulbs..... 30
30. (33) Amphids circular or nearly so; pharynx compound, much elongated 31
31. (32) Chitinous external marking of amphids circular..Plectus, Bastian
32. (31) Chitinous external marking of amphids not circular..... ..... Chronogaster, Cobb
33. (30) Amphids apparently absent..... 34
34. (37) Œsophagus with two more or less well developed bulbs; males with a bursa..... 35
35. (36) With no "diggers" on head.....Rhabditis, Dujardin
36. (35) With dorsal and ventral "diggers" on head....Diploscapter, Cobb
37. (34) Œsophagus with only one well developed bulb; males without bursa 38
38. (39) Pharynx long and narrow.....Rhabdolaimus, de Man

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39. (38) Pharynx not long and narrow..... 40
40. (41) Striæ not resolvable into rows of dots.....Cephalobus, Bastian
41. (40) Striæ resolvable into rows of dots, altered on lateral fields ..... Teratocephalus, de Man
42. (29) Œsophagus without bulbs..... 43
43. (46) Pharynx none 44
44. (45) Amphids spiral.....Aphanolaimus, de Man
45. (44) Amphids circular or ellipsoidal.....Tripyla, Bastian
46. (43) Pharynx present 47
47. (48) Cavity relatively large, amphids very small..... ..... Prismatolaimus, de Man
48. (49) Cavity small, amphids usually well developed..... 49
49. (50) Form of cavity conoid, open in front; circular amphids considerably behind it.....Monhystera, Bastian
50. (49) Form of cavity various, closed in front; amphids nearly opposite it 51
51. (52) Lateral organs inconspicuous.....Trilobus, Bastian
52. (51) Lateral organs more or less conspicuous spirals or circles.. ..... Anonchus, Cobb

VII. Literature Cited.

The following specific descriptions have been prepared by Dr. N. A. Cobb, Department of Agriculture, Washington, D. C., who examined the collection with the view of adding to the completeness of the key he is preparing for the identification of the American free-living freshwater nematodes.

1. **Tylencholaimellus n. g. diplodorus**, n.sp.

				47		
.6	6.6	12.	-M-	97.		
1.1	2.2	3.6	4.7	2.7	.8	mm.

The thin transparent layers of the colorless, naked cuticle are traversed by exceedingly fine transverse striæ visible for the most part only in the subcuticle. Longitudinal striæ exist throughout the body, probably associated with the attachment of the muscle cells to the subcuticle. The conoid neck becomes convex-conoid near the head which is rounded and not set off in any way. On the outer margin of the head there are six minute flattish conoid papillæ which when seen in profile slightly interrupt the outer contour of the head. Surrounding the mouth opening there are what appear to be similar papillæ, but they are very inconspicuous so that at first glance the head appears to have only a single circlet of cephalic palillæ, namely, the posterior. The vestibule is very narrow, the lips closing together over the apex of the spear, which apparently is located normally a distance twice as far from the anterior extremity as the posterior circlet of papillæ. The main portion of the spear is one-fifth as wide as the corresponding portion of the head. This is followed by a "hilt" half as long as the main shaft, and half as wide again, ending in a short, three-bulbed base fully one-third as

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wide as the corresponding portion of the neck. The entire length of the spear is about four times the width of the lip region measured opposite the posterior circlet of papillæ. The anterior portion of the spear is more strongly chitinized than the posterior portion. It has a dorsally oblique opening similar to that seen in Dorylaimus, and presents the striking peculiarity of *having an extra piece of chitin on the dorsal side.* This extra piece is bowed outward very slightly so that there is a space between it and what would be regarded as the normal dorsal wall of the spear,—that is, the wall which comes next to the lumen, through which the food must pass. It is this peculiarity which gives rise to the specific name. The œsophagus begins at the base of the spear as a tube about one-fourth as wide as the corresponding portion of the neck and continues to be narrow to near the posterior portion of the neck. At the beginning of the posterior fifth of the neck, or thereabouts, the œsophagus suddenly enlarges to produce an elongated cardiac swelling or bulb two-thirds as wide as the base of the neck and two and one-half times as long as wide. The lining of the œsophagus is very faint throughout its length. There is no very distinct cardia. The intestine, which is separated from the œsophagus by a distinct constriction, becomes at once three-fourths as wide as the body. Its cells contain small scattered fairly numerous yellowish granules which are not disposed so as to give rise to any tessellation. The appearance by transmitted light is that of a firmament in which the stars are unusually close together.

The tail of the male begins to taper from some little distance in front of the slightly elevated anus and is conoid to the rounded terminus which has a diameter one-third as great as that of the base of the tail. No papillæ or hairs occur on the tail. The supplementary organs are two in number. One occurs a short distance in front of the anus opposite the junction of the distal and middle thirds of the spicula. The second is nearly twice as far in front of the anus as the proximal ends of the spicula. The supplementary organs are simple in character, consisting of very low flat cones which interfere but little with the ventral contour. The posterior is perhaps double, that is, consists of two papillæ side by side close to the ventral line. The two equal arcuate spicula are one and two-thirds as long as the anal body diameter. They are simple in character and are made of a rather frail framework. They are widest toward the proximal extremity where the greatest width is one-fourth as great as that of the corresponding body diameter. They taper toward the proximal end from this widest part, and the proximal end is almost imperceptibly cephalated by expansion. No very distinct accessory pieces have been seen, though the lining of the sheath in which the spicula glide is a little more pronounced toward the anus. The faintest possible traces of very oblique copulatory muscles have been seen. There are two small testicles outstretched in opposite directions after the manner of Dorylaimus. The blind end of the anterior one is one and one-half times as far behind the base of the neck as this latter is behind the anterior extremity. Nothing is known concerning the renette.

Locality: Maple River, Mich.

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2. **Actinolaimus propinquus,** n.sp. $\frac{.6\ \ 6.7\ \ 24.\ \ '47'\ \ 27\ \ 91.}{.8\ \ 1.5\ \ 1.9\ \ 2\ \ 1.2}$ 2.4 mm.*

The thin layers of the transparent, colorless, naked cuticle appear to be destitute of transverse striations. If any occur they must be exceedingly minute. Faint longitudinal striations exist throughout the length of the body, and are most readily visible outside the borders of the lateral fields. The posterior portion of the neck is cylindroid; toward the head it becomes conoid. The subtruncate head is set off by a very broad, almost imperceptible constriction. The contour of the front of the head is approximately flat. This flat portion occupies about two-thirds of the diameter of the front of the head, and from its margin the head slopes at an angle of forty-five degrees to join the short subcylindrical portion of the head opposite the anterior part of the pharyngeal cavity. The lips are thoroughly amalgamated, and the two blunt angles caused by the oblique marginal surface are the locus of the two circlets of labial papillæ which do not extend out so as to materially break the contour, but are seen in optical section as refractions rather than as elevations. The innervations are obscure. Amphids are present in the form of somewhat elliptical markings, the plainest of which is a refractive chitinous curve on the surface of the head opposite the anterior portion of the collar of the spear. The width of these markings is about one-fourth to one-third as great as that corresponding portion of the head. When the amphids are seen dorso-ventrally they have the form of narrow slits leading inward and backward from a distinct narrow groove opposite the base of the lips, that is to say, opposite or slightly behind the bottom of the anterior portion of the pharyngeal cavity. The mouth opening is circular and fits the spear. Surrounding the mouth there is a somewhat discoid structure which is separated from the outer portion of the wall of the head by a circular groove as in *Actinolaimus radiatus.* The outer wall of this shallow groove is very finely longitudinally striated. The disk-like structure represents the inner portion of the lip region and is arched over the anterior part of the pharynx which is more or less globoid in form, and one-half as wide as the corresponding portion of the head. At its base there are six chitinous processes which surround the spear and serve to guide it in action. These project into the spheroidal chamber so that its base, instead of being flat or concave, is really convex or conoid toward the lips. The optical section of this portion of the cavity is therefore that of a somewhat broad crescent rather than circular. The lateral walls of this cavity when seen in optical section sometimes present appearances leading to the conclusion that it is minutely dentate in some such way as is the wall of the pharynx of some species of *Mononchus.* The observations on this point, however, are not yet sufficiently decisive. At a distance from the anterior extremity equal to about two-thirds of the radius of the front of the head is found the beginning of the

* Formula derived from a single specimen which apparently had lost a short portion of the tail.

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guiding collar for the spear, which is compound, wider posteriorly than it is anteriorly. Anteriorly it is but slightly wider than the spear, and there is a narrow, refractive chitinous element or ring clearly indicating the anterior border of the collar. The substance of the collar behind this border is practically cylindrical, although it diminishes slightly in diameter to a little in front of the middle of the collar, where it is flared and receives a second element inside the space formed by the flare. This second element is itself flared, though it is smaller than the first, and is continued backward and ends rather indefinitely. Both these expansions, or "capes," are margined with refractive chitinous rings. Measuring to the limit of the second flare, which is indicated by a chitinous ring similar to that which indicates the anterior limits of the collar, it may be said that the collar is about as long as it is wide in its widest part, namely, about twice as wide as the spear. This latter is fully one and one-half times as long as the head is wide. It is slightly swollen just behind the oblique dorsal aperture. In its posterior three-fifths the spear is cylindrical, while in the anterior two-fifths it tapers to an acute point, the ventral contour being slightly arcuate, while the dorsal contour is nearly straight. The diameter of the shaft of the spear is about one-fifth as great as that of the corresponding portion of the head, perhaps a little less. The walls of the spear are well chitinized. Following the spear is a tubular, rather indefinite portion, about three-fourths as long as the spear, which joins a fusiform swelling about one-half as wide as the corresponding portion of the neck and about as long as the spear, through which the lumen continues to be only slightly less narrow than in the spear itself. The fibers in this fusiform portion extend forward as well as outward. Behind this fusiform part the œsophagus is a tube nearly one-third as wide as the corresponding portion of the neck. It continues to have this diameter, although it varies a little, to a short distance in front of the nerve ring, where there is an obscure swelling of very faint character. Behind this swelling, the œsophagus for a short distance, where it passes through the nerve ring, is about one-fourth as wide as the corresponding portion of the neck, or a little less. Behind the nerve ring it expands gradually to a point where there is a break in the musculature. At this point the œsophagus is nearly two-fifths as wide as the corresponding portion of the neck. It continues to have this diameter for a distance nearly equal to two of the corresponding body diameters and then rather suddenly enlarges at a point where there is a second break in the musculature. Thence onward the œsophagus is cylindrical and nearly two-thirds as wide as the corresponding portion of the neck. For a short distance near the base of the œsophagus the internal tissues stain differently from those farther forward. They take the carmine more strongly. This portion of the œsophagus is one and one-half times as long as it is wide. It is not set off from the rest of the œsophagus by any striking difference in the internal structure. The lining of the œsophagus is a distinct feature, especially in the enlarged cylindrical portion, where its optical expression consists of several marked refractive,

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parallel, longitudinal lines occupying fully one-fourth of the optical longitudinal section. The rather broad conoid cardia is two-thirds as long as the body is wide. The cardiac collum is about one-third as wide as the body and presents the peculiarity that the lining of the œsophagus extends into it a short distance. The constriction between the œsophagus and the intestine is not only deep but rather broad, and one or two special spherical cells are to be seen in this region. The thick-walled intestine becomes at once about three-fifths as wide as the body, and is composed of cells of such size that a considerable number are required to build a circumference, probably about eight. These cells contain numerous brownish-yellow granules of variable size, the largest having a diameter about one-half as great as the internal diameter of the spear. The tail begins to taper from some little distance in front of the anus and tapers at the same rate behind the anus. It continues so to taper until near the extremity, which is sometimes blunt, sometimes conical, and sometimes subacute. For some little distance in front of the terminus, namely, a distance fully equal to the length of the anal body diameter, the tail is cylindrical and not more than one-twelfth to one-twentieth as wide as at the anus. This latter is very slightly raised in that the posterior lip is slightly elevated. From it the conspicuous rectum, which is lined with refractive chitin, and is fully twice as long as the anal body diameter, extends inward and forward. Anal muscles are present and rather conspicuous, as are also rather numerous glandular cells. The pre-rectum is about five times as long as the corresponding body diameter and about three to four times as long as the rectum. The pre-rectum differs from the rectum in being smaller in diameter, and its cells do not contain the brownish-yellow granules seen in those of the intestine. The lateral fields are distinct and nearly one-third as wide as the body. Nothing is known concerning the renette. The nerve ring surrounds the œsophagus somewhat squarely. From the rather small vulva the vagina leads inward at right angles to the ventral surface about half way across the body, where it joins the two symmetrically placed uteri. The reflexed ovaries, which taper toward the blind end, reach three-fourths of the way back to the vulva and contain about two dozen developing ova, most of which are arranged in single file and are more or less compressed into disk-like form. The eggs are ellipsoidal and somewhat longer than the body is wide and a little less than half as wide as long. The eggs are apparently deposited before segmentation.

Locality: Maple River and Beach Pools, Douglas Lake, Mich.

3. **Dorylaimus sulcatus**, n.sp. $\frac{.5\ 12.\ 33.\ Y\ 92.}{.8\ 2.5\ 3.1\ 29\ 1.7}$ 1.6 mm.

The very thick layers of the transparent, colorless, naked cuticle appear to be destitute of transverse striations. Prominent longitudinal striæ to the number of about 32 are found throughout the length of the body except on the lateral fields where for a distance equal to about one-fourth of the body-width, they are absent. The cuticle becomes thinner near the lips. The

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neck is conoid, tapering more rapidly toward the truncate head which bears a lip region set off by an almost imperceptible, broad constriction. The lips are thoroughly amalgamated, and each is armed with two plainly innervated papillæ, one on the outer surface and outward pointing, and one on the outer margin of the front surface and forward pointing. These papillæ do not interfere with the contour of the lips. The front of the head is very slightly depressed in the middle. The pharynx is beaker-shaped, half as wide as the front of the head, and a little deeper than it is wide. The apex of the spear appears on a level with the front surface of the lips. Under these circumstances this beaker-shaped portion of the pharynx is continued as a somewhat narrower cylindrical part which is nearly filled by the spear for a distance almost equal to the depth of the beaker-shaped portion. At the base of this second narrow portion of the pharynx occurs the guiding collar of the spear which closely surrounds the spear for a distance about equal to its own width. The limits of the collar are indicated by two chitinous rings, the anterior of which rather closely surrounds the spear, the posterior and stronger of which is somewhat wider. The relatively small, somewhat stirrup-shaped amphids are just behind the outer circlet of papillæ. They are about one-third as wide as the corresponding portion of the head. Their anterior contour is a nearly straight, transverse element, from the ends of which there extend backward and inward convex contours which become indefinite opposite the base of the beaker-shaped portion of the pharynx. There are no eye-spots. The massive spear is three times as long as the front of the head is wide and tapers from base to apex. Seen in profile, the taper of the posterior three-fifths is only slight, whereas that of the anterior two-fifths is more pronounced; the contour is straight on the dorsal side, where the opening is, and slightly convex on the ventral side. The apex is acute. When seen in optical longitudinal section, the chitin forming the spear is seen to be bifurcated at the posterior margin, more particularly on ventral side. At its maximum width, namely, at the base, the spear is nearly half as wide as the front of the head and about one-fifth as wide as the corresponding portion of the neck. There is no distinct fusiform swelling at some little distance behind the spear as is often the case in *Dorylaimus*. The lumen of the spear is continued by that of the œsophagus for a distance about equal to the length of the spear; at the posterior extremity of this portion there is an obscure joint in the lining of the tube. Otherwise, there is no indication of the secondary extending element of the spear. The œsophagus begins behind the secondary element as a tube fully one-third as wide as the corresponding portion of the neck, and continues to have this diameter until it passes through the nerve ring. At this point it begins to enlarge almost imperceptibly, and finally at the beginning of the posterior third, it enlarges rather rapidly so that in this portion of the neck it occupies one-half of the diameter. At the extremity, finally, it is really a little more than one-half as wide as the base of the neck. There is a somewhat spheroidal cardia, one-fourth as wide as the base of the neck. The

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lining of the œsophagus is a very distinct feature throughout its length. In the posterior enlarged portion the lining consists of several parallel longitudinal elements occupying one-fourth of the optical longitudinal section; in the narrow tubular part the lining occupies one-third of the diameter. The rather thick-walled intestine, which is separated from the œsophagus by a fairly distinct constriction, becomes at once two-thirds as wide as the body. Its walls are composed of cells of such size that probably a considerable number are required to build a circumference, perhaps six to eight. These cells contain rather numerous brownish-yellowish granules of variable size, the largest of which have a diameter about one-third as great as the thickness of the cuticle, the smallest of which are much smaller. The granules are so arranged as to suggest a faint tessellation. Of the large granules, two to three would go side by side into the base of the spear. From the slightly depressed anus the conspicuous, chitinized rectum, which is one and one-half times as long as the anal body diameter, extends inward and forward. The pre-rectum is about three times as long as the rectum and is distinctly separated from the intestine by difference in size as well as difference in structure; it is considerably narrower. Only a short distance in front of the constriction separating the pre-rectum from the intestine, the intestine is nearly fifty per cent wider than the pre-rectum. The brownish-yellow granules characteristic of the cells of the intestine are absent from those of the pre-rectum. The tail begins to taper from some distance in front of the anus; near the anus it tapers more rapidly, and again more rapidly still behind the anus, in such fashion that the anterior three-fourths are concave-conoid; the reduction in diameter is such that at the beginning of the final fourth, the tail measures only about one-tenth as wide as at the base. Thence onward the tail tapers but very slightly to the rather blunt, colorless terminus. The posterior fourth is destitute of axial matter. The cuticle of the posterior portion of the body, though not striated in the usual way, is radially striated. These radial markings come out most clearly in optical longitudinal section, and are visible on the surface, inasmuch as the surface appears to be finely granular. These striations are so minute that they would escape observation except with high powers and under favorable conditions. Once having seen this striation in the posterior part of the cuticle, one remarks it as less plainly visible throughout the body. Nothing is known concerning the renette. The nerve ring is rather massive and surrounds the œsophagus rather squarely. Connected with it, and near by, are numerous ganglion cells, somewhat more conspicuous than is usual in this genus. From the depressed vulva, the strongly chitinized, massive vagina leads inward at right angles to the ventral surface fully half way across the body. The vagina is rather complicated in structure, consisting of central masses of structureless chitin forming a somewhat bell-shaped contour when seen in optical longitudinal section. These elements are surrounded by granular and fibrous muscular structures, and into the internal angle which the chitinous elements form near the axis there extend

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other refractive, more or less chininous elements. Small unicellular glands are to be seen on both sides of the vagina,—anterior and posterior. The uteri are two in number and symmetrically disposed. The reflexed ovaries reach half way back to the vulva, at least in immature specimens such as that from which the description is derived. At a distance in front of and behind the vulva about equal to the length of the corresponding body diameter, there are slightly depressed ventral areas in which the radial striation of the chitin is materially altered and into which there appear to pass fibrous elements from the sub-cuticle, connected with special groups of small cells, presumably nerve cells, located in the body wall.

Locality: Maple River, Michigan.

4. **Ironus minor**, n.sp. $\frac{2.2 \quad 8.1 \quad 21.9 \quad 35.9 \quad 51.8 \quad 90.2}{1. \quad 1.5 \quad 1.7 \quad 1.9 \quad 1.1}$ 1.6 mm.

The moderately thick layers of the transparent, colorless, naked cuticle appear to be traversed by exceedingly fine transverse striæ. The conoid neck ends in a slightly expanded, rounded head whose chitinous elements are unusually refractive. On the outer surface of the head at a distance from the anterior extremity about equal to the radius of the head there is a circlet of four, equal, arcuate, spreading cephalic setae, one-third as long as the corresponding portion of the head is wide. There are three lips each armed with a highly refractive, arcuate-conoid, acute, inward-pointing tooth with a somewhat forward pointing apex. These teeth, which are nearly half as long as the head is wide, are arranged opposite each other near the mouth opening and are doubtless capable of being everted, though they have not been seen in the everted position. In growing specimens additional sets of these teeth are often seen at a distance from the anterior extremity about equal to two to three head widths. Immediately behind the vestibule, which is the narrow passage leading between the teeth, the pharynx is wider than it is farther back. At this point, immediately behind the base of the lips the pharynx is one-half as wide as the corresponding portion of the head. It soon narrows, so that at a distance from the anterior extremity equal to the diameter of the head, it is less than one-third as wide as the corresponding portion of the head. Thence on, it is of uniform diameter, tubular and slightly sinuous to the truncate end. The walls of the pharynx are distinctly chitinized and are surrounded with little tissue. The œsophagus begins at the base of the pharynx as a tube two-thirds as wide as the corresponding portion of the neck and very gradually expands as it passes backward, so that finally it is two-thirds as wide as the base of the neck. The lining of the œsophagus is a distinct feature throughout its length and generally appears in optical, longitudinal section as a series of two or three parallel longitudinal lines, occupying about one-eighth of the optical section. There is a flattish-hemispherical cardia about half as wide as the base of the neck. The vaguely-tesselated intestine, which becomes at once about two-

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thirds as wide as the body, is separated from the œsophagus by a distinct constriction. It is thick-walled and is composed of cells of such size that probably but few are required to build a circumference. These cells contain scattered, yellowish-brown granules of variable size. From the inconspicuous but slightly elevated anus, the rectum, which is one and one-half to two times as long as the anal body diameter, extends inward and forward. The rectum is separated from the intestine by a fairly definite pyloric constriction. The tail tapers from some distance in front of the anus and continues to taper behind the anus at about the same rate to the nearly hair-fine terminus, which is destitute of a spinneret. The longitudinal fields appear to be one-third as wide as the body. The nerve ring surrounds the œsophagus somewhat obliquely. Nothing is known concerning the renette. From the elevated vulva, the well developed vagina leads inward at right angles to the ventral surface half-way across the body, where it joins the two symmetrically placed uteri. The reflexed, tapering ovaries reach about half-way back to the vulva and contain about eight developing ova. The moderately thin-shelled, smooth eggs are much elongated and about five times as long as the body is wide, and about one-sixth as wide as long. They appear to be deposited before segmentation begins.

Locality: Beach pools and bogs, Douglas Lake, Michigan.

					19.		
5. **Mononchus lacustris**, n.sp.	2.1	7.7	27.	64.	95.	1.7 mm.
	1.8	2.1	2.4	2.7	1.8	

The thin layers of the transparent, colorless, naked cuticle appear to be destitute of transverse striations. If any are present they must be exceedingly minute. The cylindroid neck tapers slightly toward the head, which bears a very slightly expanded lip region composed of six confluent lips, each bearing two papillæ, one on the outer surface and outward pointing, and one on the front surface halfway between the margin and the body axis, and forward pointing. This inner circlet of papillæ is composed of members of somewhat larger size than those of the outer circlet. Both are flattish conoid in shape, and impart to the contour of the lips a somewhat angular appearance. The thick lips are arched together over the goblet-shaped pharynx, which has a depth equal to the diameter of the head, measured opposite the base of the pharynx. At its widest part, a short distance behind the lips, opposite the apex of the dorsal tooth, the pharynx is three-fifths as wide as the corresponding portion of the head. Thence backward it tapers rather regularly to near the base, where it tapers a little more rapidly in that part which extends into the anterior end of the œsophagus. The walls of the œsophagus are well chitinized. The dorsal tooth springs from near the middle of the dorsal side of the pharynx, and extends inward and forward. The apex is forward pointing. At its greatest width the tooth is about one-third as wide as the corresponding portion of the pharynx, though it projects forward so that its apex lies opposite the axis of the head. The

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axis of the dorsal tooth is not straight, but slightly curved, that is to say, has the form of a line of beauty. Surrounding the œsophagus opposite the dorsal tooth there is a band of minute chitinous teeth on the wall of the pharynx arranged in transverse series. Where these teeth are most numerous there are at least six transverse rows. They begin near one lateral line and extend around the ventral side of the pharynx to the other lateral line. Viewed at a certain angle, which is very near the lateral view, these teeth appear, on a surface inspection, to occupy an elongated elliptical area, and with low powers one might easily mistake one of these areas for the outer expression of one of the amphids. Behind this area of minute rasp-like teeth there are two small projections from the inner surface of the pharynx, which are undoubtedly rudimentary teeth of the same character as the large dorsal teeth. These are several times larger than the minute teeth just mentioned, and are located opposite the base of the large dorsal tooth just behind the rasp-like area. They are practically submedian in position, one near each ventrally submedian line. The inconspicuous amphids are on the lips just behind the outer papillæ. They are somewhat circular in contour, one-fifth as wide as the lip-region, and only their anterior contours are definite. There are no eye-spots. The œsophagus receives the base of the pharynx, and is at that point almost imperceptibly enlarged. A short distance behind the pharynx the œsophagus is nearly three-fifths as wide as the corresponding portion of the neck. Thereafter it diminishes very slightly, until after it passes through the nerve-ring, when it begins to increase, and continues gradually to increase throughout the remainder of its length. It finally becomes two-thirds as wide as the base of the neck. The lining of the œsophagus is a very distinct feature throughout its length, more particularly in the anterior part, where it occupies about one-fourth of the longitudinal optical section, and finds optical expression in three refractive parallel lines. There is no very distinct cardia. The intestine, which is set off from the œsophagus by a distinct constriction, becomes at once about two-thirds as wide as the body. It is composed of cells of such a size that six to eight posteriorly, and ten to twelve anteriorly, are required to build a circumference. These cells are packed with minute granules of rather uniform size, which, together with refractive cell walls, give rise to an indistinct tessellation. From the raised anus the conspicuous rectum, which is about as long as the anal body diameter, extends inward and forward. The conoid tail tapers from the anus and ends in a subtruncated terminus, containing a single somewhat depressed axial pore, which forms the outlet for the secretions of the three caudal glands. These latter lie in a tandem series, one opposite the rectum, and the other two immediately behind, the posterior cell being slightly the smallest, and having its limits near the middle of the tail. The ducts form a conspicuous feature, and together occupy about one-third the diameter of the middle of the tail. They are slightly expanded posteriorly, so that one may with correctness speak

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of the presence of ampullæ. The lateral fields are about one-third as wide as the body. From the slightly elevated vulva the vagina extends forward at right angles to the ventral surface nearly halfway across the body, where it joins the two symmetrically placed uteri. The reflexed ovaries reach nearly three-fourths the distance back to the vulva, and contain about a dozen developing ova, arranged for the most part somewhat irregularly. The distal end of the ovary tapers rather rapidly. Judging from the size of the ripe ova the eggs are nearly twice as long as the body is wide, and not much more than one-third as wide as long. Nothing is known concerning the renette. The nerve-ring surrounds the œsophagus somewhat squarely. The single specimen examined contains the remains of an organism that has been swallowed. This may be the skin of another nematode.

Locality: Beach Pools, Douglas Lake, Michigan.

6. **Cyatholaimus fluviatilis** n.sp. $\frac{1.7 \quad 9.2 \quad 16. \quad 21 \quad '49.' \quad 86.}{2. \quad 2.6 \quad 2.7 \quad 3.9 \quad 2.3}$ .9 mm.

The thin layers of the transparent, colorless, naked cuticle are traversed by exceedingly fine transverse striæ, resolvable with the very greatest difficulty into rows of dots which are not modified on the lateral fields. The conoid neck ends in a rounded head which is not set off in any way. The lip region is divided internally into about twelve parts, whose refractions give rise to a striated appearance of the vestibule extending backward from the front of the head to near the apex of the dorsal tooth. When the mouth is partially open each of these elements has a triangular contour, the bases being adjacent to a sinuous, refractive, chitinous line encircling the base of the lips on the inside of the vestibule. The pharynx is open and somewhat elongated. Near the base it is about one-fourth as wide as the base of the head. A little farther forward it is 50% wider; in this part the thumb-shaped dorsal tooth is found. This tooth springs from the dorsal side of the pharynx and extends inward at an angle of approximately 30° with the axis of the head. It is slightly arcuate, so that its apex is directed nearly forward. The wall of the pharynx is fairly well chitinized. There are ten cephalic setæ on the outer margin of the head opposite the apex of the dorsal tooth, two on each submedian line, and one on each lateral line. These setæ are about equal in length throughout the series,—about one-third as long as the corresponding diameter of the head. The amphids are so located that their posterior margins are a little in front of the base of the pharynx; they consist of spirals of a little more than two winds, and are about one-third as wide as the corresponding part of the head. The œsophagus surrounds the pharynx without being enlarged. It begins at the base of the pharynx as a tube fully two-thirds as wide as the corresponding portion of the neck, and retains this diameter throughout most of its length, but finally expands to form an obscure, ellipsoidal bulb or pseudo-bulb, five-sixths as wide as the base of the neck. The lining of the œsophagus is a

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fairly distinct feature throughout its length. There is no distinct valve in the pseudo-bulb. The moderately thick-walled intestine is separated from the œsophagus by a very deep and distinct constriction and becomes at once two-thirds as wide as the body. The cells composing the intestine are of such size that about eight to ten are required to build a circumference. They contain scattered granules of variable size, the largest of which have a diameter such that they would slip inside the dorsal tooth. From the inconspicuous anus the *very unusually long rectum* extends inward and forward. The rectum is separated from the intestine by a distinct pyloric constriction. The entire length of the rectum is three to four times as great as the anal body diameter. The tail is conoid from the anus to the terminus, whose diameter is about one-fifth as great as that of the base of the tail and bears a plain, conoid, apparently unarmed spinneret. There do not appear to be any setæ on the tail. The lateral fields are about one-half as wide as the body and are composed of cells of large size arranged somewhat irregularly in two rows. From the slightly elevated vulva the chitinized vagina leads inward at right angles to the ventral surface nearly half-way across the body, where it joins the two symmetrically placed uteri. The reflexed, tapering ovaries reach half way back to the vulva and contain comparatively few developing ova. The thin-shelled eggs have been seen in the uterus one at a time and are three times as long as the body is wide, and somewhat less than one-third as wide as long. They are apparently deposited before segmentation begins.

Locality: Maple River, Michigan.

7. **Chromadora inornata**, n.sp. $\frac{3.2 \quad 10.8 \quad 19. \quad 49. \quad 90.}{3.2 \quad 4.8 \quad 5.2 \quad 6.4 \quad 2.4}$ .5 mm.

The thin layers of the transparent, colorless, naked cuticle are traversed by very fine striæ which do not appear to be further resolvable. It should be noted, however, that the examination was confined to a single balsam specimen, and that a more complete observation may perhaps show the striæ to be further resolvable. Along the lateral field are faint longitudinal striæ, beginning near the middle of the neck and extending on to the tail. Of these there are two double lines quite near together, occupying a space probably not greater than one-twelfth of the diameter; outside these, other fainter lines. No traces of cephalic setæ have been seen, but, as the description is derived from a single specimen, it is barely possible that they are present and have been broken off. Other specimens in the same lot being in fairly good preservation, it seems likely that there are no cephalic setæ. Unfortunately this specimen is dirty at the head end, so these matters have to be left in uncertainty. The conoid neck ends in a somewhat rounded head which is not set off in any way. In the specimen examined the lips are drawn together and the dorsal tooth is located at a distance from the anterior extremity nearly equal to the radius of the front of the head. Under these circumstances the lips are closely surrounded by a narrow vesti-

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bule. The number of lips remains uncertain, but there are at least six, and probably may be twelve. The vestibule, however, does not appear to be striated. That portion of the pharynx behind the lips and in front of the dorsal tooth is small, not more than one-fourth as wide as the corresponding portion of the head. The narrow portion behind the dorsal tooth is tubular or prismoid and extends backward to a point removed from the anterior extremity a distance slightly greater than the diameter of the head. This portion of the pharynx is only about one-eighth as wide as the corresponding portion of the head. The dorsal tooth is small, rather narrowly conoid, acute, and forward pointing, and is located at the body axis. Its altitude is about equal to the width of the posterior portion of the pharynx. There are no eye-spots. No amphids have been seen. There is a fairly distinct pharyngeal swelling, whose presence is indicated not so much by its size as by the fact that it is separated from the œsophagus by a difference in internal structure; the fibers composing it are arranged in longitudinal manner. Beginning at the base of the pharynx the œsophagus is about three-fifths as wide as the corresponding portion of the neck. It continues to have this diameter until the middle of the neck, where it diminishes very slightly in diameter and then passes through the nerve ring. Just in front of the cardiac bulb, its width is about two-fifths that of the corresponding portion of the neck. The cardiac bulb is prolate and five-sixths as wide as the base of the neck. It does not contain any distinct valve, or if it does the valve is hidden by the radiating muscles. The bulb is divided into subequal parts by a break in the musculature slightly behind the middle. There is no distinct cardia. The lining of the œsophagus is a fairly distinct feature throughout its length. The intestine, which is separated from the œsophagus by a distinct constriction, becomes at once three-fifths as wide as the body, and the width of the cardiac collum is about one-half as great as that of the base of the neck. The intestine is composed of cells of such size that probably two to three are required to build a circumference. These contain rather numerous small granules of uniform size. The anus is nearly continuous, but the posterior lip is very slightly elevated. The rather refractive rectum which is somewhat longer than the anal body diameter extends inward and forward. There is a fairly distinct pyloric collum. The tail tapers from some little distance in front of the anus and continues to taper behind the anus at the same rate to the terminus which bears an elongated, conical, truncate, unarmed spinneret, whose length is considerably greater than that of the diameter of its base. This latter is about one-eighth as wide as the base of the tail. The three caudal glands are located in tandem series in the anterior two-fifths of the tail. Their nuclei are placed toward the dorsal side. The cells themselves are contiguous and are rather broadly saccate. The lateral fields are one-third as wide as the body. The renette is located a short distance behind the base of the neck and presses the intestine rather sharply to one side. It is about one-half as long as the body is wide and one-half as wide as long. The location of the excretory pore remains uncertain, but it

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seems likely that it is near the lips. The nerve ring surrounds the œsophagus somewhat squarely. The female organs are double and symmetrically reflexed. Nothing is known concerning the size, number and form of the eggs, as the description is derived from a single, immature female.

Locality: Maple River and Bessey Creek, Douglas Lake, Michigan.

NOTE—This species bears a general resemblance to Chromadora ōrleyi, de Man, but ōrleyi has a striated vestibule and cephalic setæ, and also appears to have a somewhat more elongated pharynx. The discovery of the male might show other differences.

8. **Ethmolaimus gracilicaudatus,** n.sp. $\frac{2.8\ \ 10.\ \ 17.5\ \ \text{'50.'}\ \ 84.}{2.\ \ \ 3.\ \ \ 3.2\ \ \ 3.5\ \ \ 2.7}$ .6 mm.

The thin layers of the transparent, colorless cuticle are traversed by fine striæ, resolvable with difficulty into rows of minute dots which are not modified on the lateral fields. Short, scattered and very inconspicuous hairs are found on the body. These are usually of a length not much greater than the thickness of the cuticle. The conoid neck ends in a rounded head which is not set off in any way. On the outer surface, removed from the anterior extremity a distance nearly equal to the length of the radius of the head, there are four very slender, spreading cephalic setæ, each half as long as the head is wide. There are scattered hairs to be found on the neck, seldom more than one-fourth as long as the corresponding body diameter. The amphids consist of spirals of a little more than one wind, placed opposite the middle of the posterior half of the pharynx and each about one-third as wide as the corresponding portion of the head. The anterior portion of the pharynx is broadly conoid or cup-shaped, and at the base of this part the forward pointing dorsal tooth is seen lying in the axis of the head. This anterior portion of the pharynx at its widest part is half as wide as the head, and is about two-thirds as deep as wide. Extending backward from this is a comparatively definite rather uniform, narrow tubular portion of the pharynx whose walls are well chitinized and which *ends suddenly* at the point where the œsophagus proper begins. The pharynx is surrounded by a distinct pharyngeal bulb which is set off from the œsophagus by a distinct constriction. The pharyngeal bulb is three-fourths as wide as the head. The anterior portion of the pharynx is surrounded by minute more or less confluent lips, too small to be accurately enumerated. It is not unlikely that there are twelve of them. They do not materially break the comparatively rounded contour of the front of the head. The dorsal tooth has a length about equal to one-sixth the diameter of the head. It is somewhat blunt and the width of its base is nearly as great as its altitude. The œsophagus begins as a tube two-thirds as wide as the corresponding portion of the neck and continues to have this diameter until it expands to form the prominent ellipsoidal cardiac bulb which is five-sixths as wide as the base of the neck. The bulb contains a rather obscure, apparently rather simple, fusiform valvular apparatus about one-fourth as wide as itself. The lining of the œsophagus

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is not a very distinct feature. The anterior portion of the intestine, which is separated from the œsophagus by a very distinct constriction, is slightly different in histological structure from that portion which immediately follows. The portion thus characterized is only about half as long as it is wide. The intestine joins the middle of the posterior surface of the cardiac bulb, and the cardiac collum is one-third as wide as the base of the neck. The intestine becomes at once about two-thirds as wide as the body, but in the anterior part is pushed to one side by the renette cell, so that immediately behind its beginning it is only about one-third as wide as the body. Its cells contain minute granules of rather uniform size, not distributed in such a way as to give rise to any tessellation. The number of cells required to build a circumference is few, perhaps only two. The lining of the intestine, at any rate in the posterior part, is refractive, so that the narrow, sinuous lumen is rather readily seen. The tail tapers from in front of the anus, but only slightly. Behind the anus it tapers more rapidly, and rather uniformly in the anterior three-fourths. The posterior fourth is rather slender and tapers but little. The spinneret is very narrow and elongated-conoid, slightly blunt at the end, and is armed with exceedingly slender, rather short setæ. The caudal glands are arranged in a close tandem series in the anterior fourth of the tail. From the anus, only the posterior lip of which is raised, the slightly chitinized rectum, which is about as long as the anal body diameter, extends inward and forward. The lateral fields are one-third as wide as the body. The nerve ring surrounds the œsophagus somewhat obliquely. The renette is a cell half as long as the body is wide and apparently two-thirds as wide as long, located a short distance behind the base of the neck. The position of the excretory pore remains to be discovered. From the rather inconspicuous vulva, the chitinized vagina leads inwards at right angles to the ventral surface half way across the body, where it joins the two symmetrically placed uteri. The reflexed ovaries reach well back toward the vulva. This description is derived from a female specimen in an unsatisfactory state of preservation, and these details are subject to revision. Nothing is known concerning the size, number and form of the eggs.

Locality: Maple River, Michigan.

The female of this species at any rate presents many of the characters belonging to Dr. de Man's genus *Ethmolaimus,* but possesses distinct spiral amphids which could hardly have been overlooked in the type species of *Ethmolaimus.*

					53.		
9. **Aphanolaimus viviparus,** n.sp.	.5	9.	16.	'48.'	87.	1.2 mm.
	.3	1.5	1.8	3.	1.5	

The moderately thick layers of the transparent, colorless, naked cuticle are traversed by 700 plain transverse striæ which are not further resolvable and are not modified on the lateral fields, but which are interrupted there by a single, distinct, refractive wing. The contour throughout is somewhat crenate.

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The conoid neck ends in a rounded head whose lip region is set off by an almost imperceptible expansion. At the base of the lip region, at a distance from the anterior extremity nearly equal to the radius of the lip region, there are four well-developed, submedian, spreading, rather flexible, slightly-tapering, cephalic setæ, each about one and one-half times as long as the head is wide. The three exceedingly small lips project a little from the middle of the front of the head, and lead directly to the œsophageal passage. There does not seem to be any distinct pharynx. The amphids are so located that their anterior borders are opposite the bases of the cephalic setæ. They sometimes appear to be subcircular, a little longer than they are wide, and appear to be about two-thirds as wide as the corresponding portion of the head. In some specimens they may be clearly seen to be open spirals of about one and one-half winds. Their contours are distinctly refractive, and the tissues just outside them stain a little more strongly. The striæ, which are so prominent on the neck as to cause a distinct crenate contour, become less distinct opposite the posterior borders of the amphids. There seem, nevertheless, to be about three striæ opposite the amphids. There are no eye-spots. The œsophagus begins as a tube about one-half as wide as the base of the head and expands but little and very gradually. Toward the end it becomes half as wide as the corresponding portion of the neck and then diminishes so as to be only one-fourth as wide as the base of the neck; for a short distance before joining the intestine it tapers so as to be one-half as wide as it is a short distance farther forward. The constriction between the œsophagus and the intestine is very broad and shallow. The lining of the œsophagus is a distinct feature throughout its length. There is no distinct cardia, but for a short distance the cells composing the intestine are smaller and stain more strongly than those farther back. The intestine gradually becomes one-half as wide as the body and is composed of cells of such size that only a few, probably three to four, are required to build a circumference. The intestine is moderately thick-walled. It is separated from the œsophagus by a distinct cardiac constriction. From the slightly raised anus the chitinized rectum, which is one and one-half times as long as the anal body diameter, extends more directly forward than is usually the case. The tail tapers from a little distance in front of the anus and continues to taper at the same rate behind it until near the end, where it is cylindrical for a short distance. The terminus is not expanded and ends in a concave-conoid, truncated, unarmed spinneret. It constitutes a short, broad apiculum. The caudal glands constitute a rather open tandem series in front of, opposite to, and behind the anus,—occupying the anterior fourth or fifth part of the tail. The lateral fields are about one-third as wide as the body. The renette cell is a much elongated cell lying opposite the cardiac constriction. It is fully twice as long as the body is wide and not much wider than the œsophagus. It possesses a distinct nucleus near its middle. The duct leading forward from it is narrow and apparently empties through the excretory pore located opposite the nerve ring. The nerve ring sur-

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rounds the œsophagus obliquely. From the almost imperceptibly elevated vulva the chitinized vagina leads inwards at right angles to the ventral surface one-third the distance across the body, where it joins the two symmetrically placed uteri. The small reflexed ovaries reach about one-fourth the distance back to the vulva and contain about a dozen developing ova arranged for the most part single file. Each of the uteri contains a half-dozen thin-shelled smooth eggs in various stages of development, the most advanced containing fully-formed embryos. It is not unlikely that the species is viviparous. Male supplementary organs 5 to 8.

Locality: Beach pools, Douglas Lake, Michigan.

					23.		
10. **Aphanolaimus communis.**	.3	'8.7	17.	'48.'	85.	1. mm.
	.6	1.5	2.5	2.7	1.2	

The rather thin layers of the transparent, colorless, naked cuticle are transversed by about 600 transverse striæ, which exist in the outer cuticle, and are of such a nature that they cause the contour to be minutely crenate throughout the length. These striæ are not further resolvable. They are interrupted on the lateral fields by a single highly refractive wing. It is probable that there are four minute, somewhat forward pointing submedian cephalic setæ, each about one-third as long as the head is wide. The conoid neck ends in a rounded head, which is not set off in any way. The mouth pore in the front of the head is exceedingly minute; it is very slightly depressed. The circular amphids are so located that their anterior borders are removed from the anterior extremity a distance about equal to the radius of the head, or a distance about equal to their own radius. They appear to be about half as wide as the corresponding portion of the head. Although they appear circular at first sight, they are probably really spiral in form, and unclosed in the anterior border. There is a very minute pharynx about one-third as wide as the front of the head, one-half as wide as the amphids. Possibly there is a chamber extending backward from this, having a length such that it extends a little farther back than the amphids, but this is somewhat doubtful. The œsophagus begins opposite the amphids as a tube nearly half as wide as the corresponding portion of the head, and expands very slightly in diameter as it passes backward, until it reaches the nerve-ring. Thereafter it is uniform in diameter. There is no cardiac swelling. The intestine is set off from the œsophagus by a distinct constriction, and in the anterior portion is not much wider than the base of the œsophagus. Gradually, however, it widens until it becomes two-thirds as wide as the body. It is relatively thin walled, and is composed of elongated cells of such a size that probably three to four are required to build a circumference. From the rather inconspicuous anus the chitinized rectum extends inward and forward. There is a distinct pyloric collum. The tail tapers from some distance in front of the rectum, and continues to taper through its anterior four-fifths. Thence onward the tail is cylindroid to the convex-conoid terminus, which

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bears a truncate conoid spinneret. The wall of the tail is relatively thick, and the caudal glands are rather small sized, and are located in a tandem series in the anterior third of the tail. The lateral fields appear to be about one-third as wide as the body. The renette is of relatively large size, and is located opposite the posterior portion of the œsophagus. It is fully half as wide as the base of the neck, and is about one-third as wide as long. Anteriorly it tapers to join the duct, which leads forward and perhaps empties near the base of the lips. The nerve-ring surrounds the œsophagus somewhat obliquely. From the slightly depressed vulva the chitinized vagina leads inward at right angles to the ventral surface fully half-way across the body, where it joins the two symmetrically placed uteri. The broad reflexed ovaries contain a dozen or more developing ova arranged somewhat irregularly. Only the distal third of the ovary is tapering. The somewhat thin-shelled eggs are fully one and one-half times as long as the body is wide, and less than half as wide as long. They have been seen in the uteri one at a time, and apparently are deposited before segmentation begins.

$$\frac{.3 \quad 10. \quad 18. \quad M \quad 92.}{.6 \quad 2.2 \quad 2.4 \quad — \quad 1.8} \ .9 \text{ mm.}$$

The tail of the male is similar in size and form to that of the female. The two equal, arcuate spicula are one and one-half times as long as the anal body diameter. They are broadest in the middle, and of uniform width for some distance in the middle part. Near the distal extremity they diminish rather suddenly in size, and thence outward they are of uniform diameter to the end. The formation of the proximal end is somewhat the same, but this portion is longer and slightly arcuate. There is no distinct cephalum. At their widest point they are one-fifth as wide as the corresponding body diameter. In front of the anus there are ten tubular supplementary organs arranged at an angle of about forty-five degrees with the body axis. These organs are about half as long as the body is wide, and are themselves somewhat arcuate. The proximal end of each is slightly cephalated. The diameter of the main portion is about the same as the diameter of the distal portion of the spicula. Each tubule ends in a slight depression on the ventral line, and is probably protrusile. The posterior member of the series is opposite the proximal ends of the spicula, and the anterior member nearly as far in front of the anus as the terminus is behind it. The ejaculatory duct is about one-third as wide as the body. The vas deferens is about half as wide.

Locality: Beach Pools, Douglas Lake, Michigan.

11. **Prismatolaimus digitatus,** n.sp. $\frac{1.1 \quad 12. \quad 26. \quad {}^{24}\text{'63'} \quad 81.}{1.2 \quad 2.1 \quad 2.3 \quad 2.8 \quad 1.9}$ .6 mm.

The rather thin layers of the transparent, colorless cuticle are traversed by 380 transverse striæ, resolvable with high powers into rows of minute elements, at any rate toward the head end. Scattered setæ are found here and there on the surface of the body. These have a length

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equal to the width of two to three annules. The somewhat cylindroid neck tapers but little, and ends in a rounded head, which is not set off in any way. On the outer surface of the head, opposite a point between the anterior and middle thirds, there is a circlet of six wide-spreading, finger-shaped setæ, each bearing a minute bristle at its end. These setæ are nearly half as long as the head is wide. The thin lips are arched together over the pharynx, and each bears a minute, forward-pointing and slightly outward-pointing papilla, which may be seen quite distinctly with high powers when the lips are which may be seen quite distinctly with high powers when the lips are brought into exact profile. The unarmed prismoid pharynx begins immediately behind the base of the thin lips and ends suddenly at a distance from the anterior extremity equal to the width of the head. The width of the pharynx is about as great as the width of the head, and it is half as long as wide. There are no eye-spots. The amphids are ellipsoidal markings about one-third as wide as the corresponding body diameter. They have their long axis located transversely, and are situated about twice as far behind the base of the pharynx as this latter is behind the anterior extremity. The œsophagus receives the base of the pharynx, and is at first two-thirds as wide as the base of the head. It continues to have about the same diameter throughout its length, but it expands somewhat posteriorly so that it becomes about two-thirds as wide as the neck. Between the œsophagus and the intestine there is a spheroidal element nearly as wide as the œsophagus. This perhaps may be regarded as a strongly developed cardia. It is set off by a constriction on both sides, particularly on the side toward the œsophagus. The lining of the œsophagus is a fairly distinct feature throughout its length. The thick-walled intestine, which is set off from the œsophagus by a distinct constriction, becomes at once about three-fourths as wide as the body. It is composed of cells of such a size that three or four are required to build a circumference. The anus is slightly raised, and the rectum, which is as long as the anal body diameter, extends inward and forward. The tail end begins to taper a short distance in front of the anus, and tapers with considerable regularity to the terminus. This latter has a width about one-eighth as great as that of the base of the tail, and appears to bear a minute apiculum, but has no spinneret. The lateral fields appear to be about one-third as wide as the body. The nerve-ring surrounds the œsophagus somewhat squarely. Nothing is known concerning the renette. From the elevated vulva the vagina leads inward and forward. The single uterus extends forward. The ovary is reflexed, and has its blind end located in the vicinity of the vulva, at least when the uterus is not occupied by an egg. Judging from the size of what appears to be a mature ovum, the eggs are considerably elongated, probably three to four times as long as the body is wide, and about one-fourth as wide as long. The ovary may contain fifteen to twenty ova, arranged single file except toward the tapering blind end.

Locality: Beach Pools and Bogs, Douglas Lake, Michigan.

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

NEW LIFE MEMBER.

The Secretary is pleased to announce that Mr. Seth Bunker Capp of Philadelphia has been elected to Life Membership in the American Microscopical Society. The Constitution provides that the 50 dollars dues of life members shall become a permanent part of the Spencer-Tolles Fund for the encouragement of microscopic research. Members can render a real service to the Society by bringing to the attention of people of means, who are interested in encouraging scientific work, this provision of the American Microscopical Society.

ENTOMOLOGICAL NOTES.

Hearing in Saturniidæ.—Turner ('14, Biological Bulletin, *27*: 325-332) reports results of a study of the auditory powers of *Saturniidæ*. From experiments on four species: *Telea polyphemus, Samia cecropia, Philosamia cynthia* and *Callosamia promethia,* the author finds evidence which seems to prove that all of these species hear. The last three species respond readily to a large range of sounds, but *Telea polyphemus* normally does not respond perceptibly to sounds. However if experimentally the moth is caused to associate some disagreeable experience with some sounds, it can be induced to respond to the latter. Responses are looked upon as expressions of emotion.

Poison Glands.—Kephart ('14, Journ. Parasitology, 1: 95-103) finds that the poisonous element of the hairs of the larvæ of the Brown-tail Moth which produces serious irritation to human skin is contained in the short barbed hairs. Specialized hypodermal cells secrete this substance, the latter gaining entrance to the blood

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through the point of the hair when it comes in contact with the skin. There is a poison gland for each papilla on a tubercle but not one for each hair.

Behavior of Insects and Spiders.—Turner ('14, Journ. Animal Behavior, 4: 394-413) gives a summary of "The Literature for 1913 on the Behavior of Spiders and Insects other than Ants." The essential results of each paper are briefly discussed. At the end of the paper the literature for the year 1913, consisting of one hundred and twenty-six titles, is listed.

Catocala Moths.—Turner and Schwarz (Biological Bulletin, 27: 275-293) presents a paper on the "Auditory Powers of the Catocala Moths." An experimental field study on several species of the genus *Catocala* has shown that several of the species observed respond definitely to certain high pitched notes of the Galton whistle but usually fail to respond to sounds of low pitch. Degree of responsiveness differs in different species. Lack of response to sounds of low pitch is not attributed to failure to hear such sounds, but it is the opinion of the authors that these moths respond "only to such sounds as have a life significance."

Olfactory Sense of Insects.—McIndoo ('14, Smithsonian Misc. Coll., 63: No. 9) presents evidence against the old view that the olfactory sense of insects is located in the antennæ and finds that in *Hymenoptera* the organs of olfaction have their seat in certain definite regions, such as the bases of the wings, the bases of the legs, the sting of the worker and queen honey bee, and the mouth parts, where aggregations of "olfactory pores" occur. Evidence is also presented in favor of the view that possibly these olfactory pores are the true olfactory organs in all insects. No evidence is found of any organs of olfaction on the antennæ.

PAUL S. WELCH.

NOTES ON MICROSCOPIC TECHNIQUE.

Euparal.—Euparal is a mounting medium composed of a mixture of camsal, sandarac, eucalpytol and paraldehyde, having a refractive index of 1.483. It is put up in two forms, the colorless and the green, the latter containing a copper salt which intensifies hæma-

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toxylin stains. The colorless is preferable when stains other than hæmatoxylin are used. Either may be obtained from G. Grübler & Co., Leipzig.

The primary advantage of this medium is that it spares delicate objects the usual treatment with absolute alcohol, since objects may be mounted in it directly from 95% alcohol. It dries rapidly so that preparations may be studied with safety at the end of 24 hours. These two points should be of interest not only to investigators, but to those who conduct classes in embryology in which students prepare their own serial sections for study. Further, cover-slips may be readily removed from old preparations by immersion in 95% alcohol for several hours.

Another useful property of euparal is its low index of refraction which is well adapted to cytological study, giving a much desired increase of visibility to delicate elements. It may also be noted that it does not bleach the stain.

During the past year I have used euparal with a variety of material and have found it uniformly satisfactory. It is superior to Canada balsam in every way.

Safranin O and Lichtgrün.—This is a staining combination especially well adapted for the study of chromosomes, and is one that should be more widely used than is the case at the present time. In many ways it is superior to Iron-Alum-Hæmatoxylin, although it may be used to good advantage in conjunction with the latter.

The Safranin should be of good quality preferably Grübler's Safranin O. The usual solvent is anilin water which may be made by shaking up 4 or 5 cc. of either anilin oil, or old discolored anilin, in 90 cc. of distilled water and filtering. Enough 95% alcohol is added to the filtrate to make a 10% alcoholic solution, in which 1 gram of safranin is then dissolved.

Lichtgrün (Grübler's Lichtgrün L. S.), the acid counterstain, is made up by adding .5 gram of the stain to 100 cc. of 95% alcohol.

The staining procedure is as follows: Sections on the slide are stained in safranin for from 4 to 6 hours. Excess of the stain is removed by washing in 35% alcohol for a few minutes. The sections are then rapidly passed up through the graded alcohols to 95% from which they are transferred to Lichtgrün in which differentia-

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tion is done. The secret of good preparations is to let the lichtgrün act just long enough to extract the excess of safranin. A little practice will enable one to tell from the general appearance of the slide held up to the light when to stop. Usually for thin sections 30 seconds is enough. It is well to move the slide about all the time it is in the lichtgrün.

The excess of lichtgrün is hastily washed off in 95% alcohol after which the slide is immersed for a second in absolute alcohol and then transferred to clove oil for one or two minutes. Before mounting the excess of clove oil may be removed by immersion in xylol. Euparal may be used as the mounting medium after xylol.

Safranin stains chromosomes and chromatin elements a deep red for which the light green background gives an excellent contrast. Many object to lichtgrün because it is not permanent. However, I have had preparations retain their brilliancy for two years or more and that certainly is long enough for most lines of work.

Hæmatoxylin-Orange G Stain for Embryos.—To those interested in a satisfactory double stain for vertebrate embryos, attention is called to the method published by J. T. Morris in the Anatomical Record, Vol. 3, 1909, under the title "A Note on Orange G counterstaining suggesting a useful method in the handling of embryonic tissue." I have found the method extremely satisfactory in staining serial sections of human embryos. It was surprising to find that it stained chromosomes in dividing cells with all the clear-cut detail of iron-alumhæmatoxylin.

H. L. WIEMAN.

University of Cincinnati, Dept. of Zoology.

METHOD OF PREPARING FLY'S TONGUE AS MICROSCOPIC OBJECT.*

A fly's tongue when properly prepared and mounted makes a very beautiful object for the microscope, but to the novice it has been more or less a difficult task. With the following suggestions the worst part of the preparation is overcome with much ease, that is, making the lobes of the tongue lie flat and evenly spread out.

We have all noticed that when the fly places his tongue on a flat surface it spreads out the lobes in a way we would best

* Presented before State Micr. Soc. of Ill., Feb. 11, 1915.

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have it on our finished mount, so we will proceed to catch it in this position.

Have ready two glass slips and place one of them at the edge of a table where the operation is to take place. Take a fly between the thumb and finger of the left hand and the other glass slip in the right hand, hold the fly's head at the edge of the glass slip on the table and he will lay his tongue out flat on the glass. When all is right, place the slip that is in the hand on top of the tongue with enough pressure to hold it and cut off the tongue close to the head. A patent clothes-pin or elastic band may be used to hold the two glass slips together till the specimen is further treated.

The slips with the tongue clamped between them at one end are next put in a small dish in which some turpentine has been placed and is allowed to remain in the turpentine bath for five hours. It may then be taken out and will be found to be quite hard and flat, with all the beautiful colorings retained. The tongue may now be mounted with great ease in turpentine balsam or benzole balsam. Fly's feet may be prepared in the same manner.

Students who have tried in vain to make a good mount of this common object will smile when they find with what ease this seemingly difficult feat may be accomplished.

OLIVER KENDALL, JR.

METHOD OF COLLECTING DIATOMS FROM SURFACE OF MUD.

To the amateur collector of diatoms, the greatest difficulty has been to gather them free from excess of sand and foreign particles. The following method will be found to be of great help in this respect, especially on the shores of tide water, and the method requires that the surface of the mud be uncovered by the tide. The spot for working is found by the presence of a brownish colored film generally in streaks or patches on the mud surface.

It has been found that by removing the film of diatoms with a spoon large quantities of sand and mud are taken up at the same time making its removal difficult in the cleaning process.

The collector is to provide himself with several squares of well washed cotton cloth, about the size of a handkerchief, and be at the ground at low tide. Take a square of cloth and carefully

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lay it down on the mud surface in a way not to include air bells, The cloth will in a few moments become wet and may then be raised by one corner first and folded up with the side that was next to the mud on the inside. After folding wrap in waxed paper and label for future reference. When ready to clean, place the cloth in a porcelain evaporating dish and cover with strong sulphuric acid and enough bi-chromate of soda to make the mass a deep reddish color. Place the dish in a sand bath over a gas stove or other source of heat, boil the mass till crystal of chromic acid appear as a scum on the surface of liquid. Remove and let cool and pour into a preserve jar partly filled with water. Let settle for at least one-half hour undisturbed, then siphon off water with a rubber tube to within one inch of the bottom of the jar, being careful not to disturb the sediment. Repeat the washing till clear from all color. The sediment may now be removed to a small bottle and examined and if a small quantity of sand is present it may be removed by whirling it with some water in the evaporating dish by means of a glass rod, and the sand will be found to pile up in the center as a dark spot. Carefully pour off the water with the diatoms suspended in it, leaving the sand in the dish.

One will be surprised how the diatoms will stick to the cloth and how little foreign matter will be collected by this method. The above method may be used in fresh water streams provided the spot is first drained.

Providence, R. I. OLIVER KENDALL, JR.

NEW METHOD OF EXAMINING STOOLS FOR EGGS.

C. M. Fauntleroy and R. Hayden (U. S. Naval Med. Bul. Jan. 1915) suggest the following method:

1. Mix thoroughly about 2 grams of fæcal matter with 5 cc. of a 2% aqueous solution of lysol in a centrifuge tube.

2. Centrifugalize at high speed for one minute, decant the supernatant liquid, and mix a fresh quantity of the lysol solution with the sediment in the tubes. Repeat this step three times.

3. Remove small portions of centrifugalized sediment with pipette; place on slide; mix a small drop of anilin gentian violet with the sediment; cover and examine.

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The authors have used the method successfully in more than a thousand cases. All eggs, hookworms and others, stand out very clearly. Everything is stained except the eggs. These appear in the natural state because their membranes resist the stain, as they would not in an alcoholic solution, which enables one to run a slide through quickly with the certainty that no eggs are overlooked.

The lysol is not essential but is used as a precaution for disinfection. The method facilitates this disagreeable work, and is so sure that much of it can be turned over to less expert assistants. The clearness of differentiation saves much time.

Abstracted by V. A. LATHAM.

A CLEARING FLUID FOR CELLOIDIN.

Dissolve 1 oz. absolute phenol-crystals in a mixture of equal parts of oils of cedar and bergamot, using gentle heat to hasten the solution. Sections may be cleared in this direct from ordinary methylated spirit. It can be used several times.

V. A. L.

BOUIN'S FLUID: A GOOD ALL-ROUND FIXING SOLUTION

Corrosive sublimate	40 grains
Water	4 ounces
Picric acid	60 grains
Formalin	8 ounces

Dissolve corrosive sublimate in hot water. When cold add picric acid, and then the formalin. Wash with water.

V. A. L.

MOUNTING ZOOPHYTES AND POLYZOA.

Fix and stain in any suitable way. Select suitable branches, place in a little vessel of oil of cloves or other clearing agent, but never let dry. Mount in sunken cell-slip without pressure or distortion. To do so *overfill* the cell, slowly slide the cover over the fluid, and put a clip on each edge of slide. If the clip bears on the center it may crack the cover, or when it is removed the cover may reassert its level and draw in a bubble of air. Larval stages of

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crustaceans, young fishes, annelids, etc., may be put up in the sunken cells. Be sure to use colorless Canada Balsam in all cases, or the deeper parts will show yellow.

V. A. L.

HENNING'S SOLUTION FOR FIXING FLIES FOR SECTIONING.

This solution fixes, and softens the chitin. Imbedding should be rapid and brief. The washing should be done with iodised alcohol. Henning's solution:

Nitric Acid (c. p.)	16 parts
Chromic (.5% sol.)	16 parts
Corrosive Sublimate (Sat. sol. in 60% alc.)	24 parts
Abs. Alcohol	42 parts
Picric Acid (saturated watery sol.)	12 parts

V. A. L.

ILLINOIS BIOLOGICAL MONOGRAPHS

Under the general series of University Studies the Biological Department of the University of Illinois issues a double number of Volume I of the *Illinois Biological Monographs*. This number is entitled "A Revision of the Cestode Family Proteocephalidæ," by Dr. George R. LeRue. There will be four numbers per volume. The third number of the first volume will be "Studies on the Comparative Anatomy and Phylogeny of the Cestode Family Anoplocephalidæ," by H. Douthitt. Subscription price is $3.00 yearly. The price of Dr. LaRue's paper is $2.00. Address Manager University Studies, 321 Natural History Hall, Urbana, Illinois.

REVISION OF THE CESTODE FAMILY PROTEOCEPHALIDÆ.

LaRue (Ill. Biol. Monog. Vol. 1, Nos. 1 and 2, 1914) presents a discussion of the Cestodes of the Family Proteocephalidæ, including the genera *Proteocephalus* (29 species), *Choanoscolex* (1 species), *Corallobothrium, Crepidobothrium,* (1 species), *Acanthotænia,* and *Ophiotænia* (1 species). These infest the cold-blooded Vertebrates. They were formerly included in the old genus *Tænia*. The chief

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genera are *Proteocephalus,* from fishes, and *Ophiotænia* from Amphibians and Reptiles.

Proteocephalids have been found in fish in Europe, North and South America, and Africa. The species of each region are peculiar, except one species common to Europe and N. America. Those of Amphibians and Reptiles have been found in North and South America, Australia, Europe, Asia, and Africa. The distribution of each of these seems narrow. None is known to occur on more than one continent.

Five species of *Proteocephalus* occur in different host species of fishes of the same genus. Four cases are known where a species may occur in members of closely allied genera. There is at least one species that may infest fish belonging to widely different families. When species have multiple hosts the host species have a continuous distribution.

The parasitic infestation of the host is determined by the food eaten. The hosts of those cestodes that have multiple hosts are alike in some of the elements of their food supply. This allows a common intermediate host.

The author believes that this family is related to and derived from marine forms of the order Tetraphyllidea. This probability coupled with the fact that this family of parasites is so well represented in fresh water fishes raises an interesting question as to how parasites, demanding as these do an intermediate host, may have evolved from Cestode parasites of marine adaptations. Reference is made to the salmons, eels, and other forms that pass back and forth from salt to fresh water as possible agents. Yet one of the salmon species that has become permanently fresh water has parasites exclusively characteristic of fresh water. This form was clearly unable to establish its marine parasites, if it had any, in the fresh water conditions, and became the host of fresh water parasites. There is no evidence that these types are succeeding in introducing marine parasites into fresh water.

Another possibility is that the intermediate host of some kind, fish or invertebrate, may have made its transition from marine to fresh water. Then some carnivorous fresh water fish with internal conditions sufficiently like those of the original species, host to the

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mature Cestode, may have become infested by adopting the new arrivals as food. This alternative seems the more plausible.

The author believes that Amphibians and Reptiles may have become infected by eating fish or invertebrate intermediate hosts containing larvæ Proteocephalids.

OPTIC PROJECTION.

Dr. Simon Henry Gage who for many years has been an active member of this Society and has twice been its President, and his son, Dr. Henry Phelps Gage, are the joint authors of a book, just issued, with the above title. The expressed object of the book is so to place before the intelligent reader the principles of optic projection, accompanied by such simple and careful directions and illustrations, that any one may become sufficiently proficient to get real satisfaction from the practise of the art.

The tremendous growth in the realization of the value of projection in all kinds of educational work and the great advances in the making of projection apparatus make the book most valuable not alone to users of such instruments, but to manufacturers and to general students of optical processes as well. The work has been done with characteristic painstaking and attention to detail. It is a record of a tremendous amount of practical manipulation and experiment with all manner of projection devices. It is safe to predict that it will become a standard reference work and handbook for the people for whom it is intended.

One of the outstanding features of the treatise is its suggestions for adapting and combining parts of standard apparatus for special uses, and thus for making economical combinations and elaborations at home.

This book ought to do much to bring closer together the manufacturer and the user of projection apparatus. The manufacturer must make allowance for the lack of optical and mechanical knowledge and skill on the part of the users, and the user must understand that the best results can only be had by understanding the principles upon which the apparatus is built.

There are fifteen chapters, with appendices, bibliography, etc. The illustrations are abundant. These consist not merely of photo-

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graphs of apparatus, but of adequate sectional drawings of details.

The principal chapter headings are as follows: Magic Lantern with Direct Current; Magic Lantern with Alternating Current; Magic Lantern for Use on House Electric Lighting System; Magic Lantern with Lime Light; Magic Lantern with Petroleum, Gas, Acetylene, and Alcohol Lamps; Magic Lantern with Sunlight; Projection of Opaque Objects; Preparation of Lantern Slides; Projection Microscope; Drawing and Photography with Projection Apparatus; Moving Pictures; Projection Rooms and Screens; Electric Currents and their Measurement,—Wiring, Control, Candle Power, etc.; Optics of Projection; Uses of Projection in Physics; Normal Vision and Eye defects.

The appendix contains an account of the origin and development of projection apparatus; makers and dealers in this apparatus; bibliography; and an extended index.

There is nothing in print which will so nearly give all he must have to the worker who must himself install his apparatus from the ground up, and control its manipulation.

Optic Projection, by S. H. and H. P. Gage. Comstock Publishing Co., Ithaca, N. Y. Illustrated, 731 pages. Price $3.00, postpaid.

THE EVOLUTION OF SEX IN PLANTS.

Under this title Dr. J. M. Coulter writes the initial number of a new Science Series to be issued by the University of Chicago. The expressed purpose of this series is to bring alike to the specialist and the layman summaries of generally accepted results in various fields of investigation with a minimum of technical details. Each issue will limit itself to the statement of a single problem.

The present topic is discussed in eight chapters, as follows:—1, Asexual Reproduction; 2, Origin of Sex; 3, Differentiation of Sex; 4, Evolution of Sex Organs; 5, Alternation of Generations; 6, Differentiation of Sexual Individuals; 7, Parthenogenesis; and 8, A Theory of Sex. The book as a whole is a luminous organization of the facts with which the teachers of Botany are familiar.

Of course plants show the most remarkable persistence of non-sexual reproduction. The lowest plants have no other method.

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Gradually the sexual method is introduced side by side with the asexual, and as a modification of it. Later, the sexual method alternates regularly with the asexual in distinct generations; and in the Bryophytes about half way up the plant kingdom, the sexual becomes the dominant generation. In all the higher plants including the Pteridophytes, there is a reduction of the sexual stage amounting to a suppression of almost every thing except the sex cells themselves, and the subordination of the gametophyte and its dependence on the asexual sporophyte. In the animals on the contrary the asexual methods disappear and the sexual reproduction stands alone in the higher forms.

The diversity of sex first shows itself in the sex cells,—ova and antherozoids. Next the organs that produce these different cells differentiate. Finally we find the plants that bear the different sex organs come to differ. The influence of the sex function does not stop, however, with the gametophyte. In the higher plants, notwithstanding the fact that the gametophytes become much reduced, the dimorphism related to sex works back into the sporophyte structures. The spores destined to produce the two types of gametophytes become differentiated; the microsporangium and microsporophyll differ from the megasporangium and the megasporophyll. And in dicœcious plants the sporophyte that finally gives rise to the male issue may differ from that which gives rise to the female.

The amateur worker with the microscope will find that this little book will make more full of meaning his studies of the lower plants.

The Evolution of Sex in Plants, by Professor John M. Coulter. University of Chicago Press, 1914. Illustrated; 140 pages. Price $1.00 net.

PSYCHOBIOLOGY

The structural biology foundational to psychology is presented by Knight Dunlap of Johns Hopkins University in a small volume recently issued. The purpose of the book is to emphasize, for the psychological student who has not had a thoro course in morphology, the details of histology and gross anatomy which are of greatest

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psychological significance. This is conceived by the author to include not merely the customary treatment of the nervous system and the sensory epithelia, but of the muscles and glands and the relation of the nerves to these. The chapter headings are:—1, The Cell; 2, The Adult Tissues of the Human Body; 3, Muscular Tissue; 4, Nervous Tissue; 5, The Afferent and Efferent Neurons; 6, The Gross Relations of the Nerves, Spinal Cord, Brain, and other Ganglia; 7, The Visceral or Splanchnic Division of the Nervous System; 8, Glands; 9, The Functional Interrelation of Receptors, Neurons, and Effectors.

This treatment of the subject will be helpful not alone to the psychologist. The biologist will find it a distinctly valuable reappraisal of the psycho-physiological values of the well known structures. The work is well done. The illustrations are well selected. If there were no other reason these would make the book a valuable handbook to the general biologist and student with the microscope. The concluding chapter is a peculiarly clear and straightforward statement of the relations of the three main portions of the human neuro-mechanism, and the general conditions of its proper activity.

An Outline of Psychobiology. By Knight Dunlap. The Johns Hopkins Press, Baltimore, 1914. Illustrated; Royal octavo, 121 pages. Price $1.25.

BIOLOGY AND SOCIAL PROBLEMS.

This is the title of the William Brewster Clark Memorial Lectures of Amherst College for 1914. They were delivered by Professor G. H. Parker. There are four lectures in the series bearing the following titles: I, The Nervous System; II, Hormones; III, Reproduction; IV, Evolution. Professor Parker has handled these interesting themes in his usual exact and lucid manner. Each of these lectures has a double value: in the first place, each assembles and summarizes the great underlying discoveries in its field in somewhat the same spirit which this journal has sought to show in its summaries of progress, altho in a more popular way; secondly, the lecturer endeavors, in the spirit of the Brewster Foundation, to apply our knowledge in these fields to the problems of man's indi-

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vidual and evolutionary responses to the environment of which he is a part.

This interesting foundation seeks to bring the discoveries of science to the problem of the conscious control by society of human improvement.

Biology and Social Problems, by G. H. Parker. Houghton, Mifflin & Co., Boston. Illustrated; 130 pages. Price $1.10, net.

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PROCEEDINGS

of the American Microscopical Society

MINUTES OF THE PHILADELPHIA MEETING

The American Microscopical Society held its thirty-fourth annual meeting in connection with the A. A. A. S. at Philadelphia, Pa., Dec. 30, 1914.

The members met for luncheon in the gymnasium of the University of Pennsylvania on Wednesday, December 30, and enjoyed greatly the social gathering for which the Society is indebted to the courtesy of the University of Pennsylvania.

The annual meeting was called to order at 2 p. m. by Vice President H. L. Shantz, Washington, D. C., in the absence of the President. Professor Henry B. Ward was elected secretary pro. tem. The letters from the Secretary, Professor T. W. Galloway, were read in full, giving a record of the work of the Society during the year, its present condition, and future possibilities. The report proved of great interest and was discussed in an appreciative manner by the members present. All were outspoken in regretting that the Secretary was unable to attend the meeting, and in appreciation of the work which he had done in behalf of the organization.

The report of the Treasurer was read and referred to an auditing committee consisting of Professors H. B. Ward and H. J. Van Cleave with instructions to print their report in connection with the report of the treasurer in the volume.

The report of the custodian was read by that official and explained in extenso. It was referred with accompanying documents to an auditing committee consisting of Doctors B. H. Ransom and R. H. Wolcott who later reported the account to be correct. This report was accepted and ordered printed. The discussion of the custodian's report was extended and interesting. It embraced an analysis of the present condition of the fund the factors in its growth which had brought it to its existing level, and of the outlook for its future extension. The Society passed a unanimous vote of thanks to the Custodian for his careful and continued efforts which have resulted so satisfactorily in the growth of the fund and expressed the hope that means might be taken in co-operation with him to increase the sum to still larger figures.

The Custodian made a full report concerning the official copy of the publications of the Society, including not only the regular Transactions, but also the incidental notices and other printed matter given out by the organi-

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zation. At the conclusion of the discussion it was voted that the official copy of the Transactions of the American Microscopical Society which was inaugurated by the Custodian and has been conducted so that it contains the full history of the organization by reason of including all notices and circulars issued, be designated the official copy of the Society and be entrusted to the Secretary for preservation. It was further voted that the copy be continued by the Secretary in the same manner as has been employed heretofore.

Inquiry was made concerning the standard rulings which are the property of the Society, and the Custodian reported that under vote passed by the Society the same were loaned to Doctor M. D. Ewell for use during a limited period. The Custodian was on motion directed to call in this and other property of the Society.

It was upon motion formally decided that the United States Bureau of Standards be designated the depository of the standard centimeter A, and other rulings property of the Society provided they were willing to accept the trust. The Custodian was directed to correspond with them with a view to ascertaining their willingness, and in case of favorable reply to place these standards in their hands with the understanding that the same may be, under proper conditions as designated by them, available for use by workers with the microscope when desired.

A nominating committee was appointed consisting of Doctor B. H. Ransom, Mr. E. Pennock, Doctor A. M. Bleile, Doctor R. H. Wolcott, and Mr. Magnus Pflaum. The committee reported the following nominations:—For President, Professor C. A. Kofoid, University of California; First Vice President, Doctor L. D. Swingle, University of Utah; Second Vice President, Doctor N. A. Cobb, United States Department of Agriculture, Washington, D. C.; for elective members of the Executive Committee, Professors J. P. Campbell, University of Georgia, L. E. Griffin, University of Pittsburgh, and Doctor H. L. Wieman, University of Cincinnati; for representatives of the American Microscopical Society on the Council of the American Association for the Advancement of Science, Doctor H. L. Shantz and Doctor R. H. Wolcott.

On motion the Secretary was instructed to cast a unanimous ballot in favor of the gentlemen nominated and they were duly elected. On motion the Society approved the action of the Secretary and authorized him to proceed with the measures inaugurated for printing and circulating the annual volume and for bringing the Society to the attention of those likely to be interested.

The Society adjourned.

HENRY B. WARD,
Temporary Secretary.

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SPENCER-TOLLES FUND.

Custodian's Report for the year 1914

Reported at Atlanta meeting		$3,819.83
Dividends received	$ 234.32	
Sale of proceedings	75.00	
Contribution	100.00	
Expense returned by Treasurer	5.50	414.82
		$4,234.65
Less Grant No. 4		50.00
Total invested		$4,184.65

GRAND TOTALS.

All contributions to date	$ 800.27	
All sales of proceedings	758.38	
All life memberships	250.00	
All interest and dividends	2,566.00	$4,374.65

LESS:

All grants to date	150.00	
All life membership dues paid	40.00	190.00
Net balance		$4,184.65

Life members: Robert Brown, dec'd.; J. Stanford Brown; Henry B. Duncanson; A. H. Elliott; John Hately.

Contributors of $50 and over: John Aspinwall; Iron City Microscopical Society; Magnus Pflaum; Troy Scientifiic Society.

MAGNUS PFLAUM, Custodian.

We, the undersigned committee hereby certify that we have carefully examined the foregoing account of Magnus Pflaum, the Custodian, and found the same to be correct.

B. H. RANSOM,
ROBERT H. WOLCOTT,
Auditing Committee.

Dec. 30, 1914.

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ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY.

December 24, 1913, to December 24, 1914.

RECEIPTS

Balance on hand from 1913	$ 265.11
Dues of old members	412.00
Dues of new members	74.00
Initiation fees	111.00
Subscriptions for volume 32	79.50
Subscriptions for volume 33	23.50
Subscriptions for volume 34	7.00
Subscriptions for other volumes	54.00
Grant from Spencer-Tolles Fund for printing N. A. Cobb's paper	50.00
Advertisers in volume 32	228.00
Advertisers in volume 33	75.00
Total	$1,379.11

EXPENDITURES

Printing Transactions, volume 32, no. 4	$ 268.81
Printing Transactions, volume 33, no. 1, 2, 3	526.74
Plates for Transactions, volume 32, no. 4	41.38
Same for volume 33, no. 1, 2, and 3	67.14
Postage and express for Secretary	46.28
The same for the Treasurer	14.21
Office expenses of Secretary, stationery stenography, etc	97.38
The same for the Treasurer	17.36
Advertising literature	19.93
Sundry expenses	.77
Contribution to Spencer-Tolles Fund for expenses of the fund	10.50
Secretary's expenses at the Atlanta meeting	25.00
Balance on hand	243.61
Total credits	$1,379.11

T. L. HANKINSON, Treasurer.

We as a committee have audited the report of the Treasurer and compared it with the vouchers and find it to be correct.

H. B. WARD,
H. J. VAN CLEAVE,
Auditing Committee.

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ORGANIZED 1878 INCORPORATED 1891

APRIL
1915

VOLUME XXXIV No. 2

George E. Coghill,

TRANSACTIONS
OF THE
AMERICAN MICROSCOPICAL SOCIETY

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EDITED BY THE SECRETARY

VOLUME XXXIV

NUMBER TWO

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DECATUR, ILL.
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Custodian: MAGNUS PFAUM.....Meadville, Pa.

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R. H. WARD, M.D., F.R.M.S., of Troy, N. Y., at Indianapolis, Ind., 1878, and at Buffalo, N. Y., 1879
ALBERT MCCALLA, Ph.D., of Chicago, Ill. at Chicago, Ill., 1883
T. J. BURRILL, Ph.D., of Urbana, Ill., at Chautauqua, N. Y., 1886, and at Buffalo, N. Y., 1904.
GEO. E. FELL, M.D., F.R.M.S., of Buffalo, N. Y., at Detroit, Mich., 1890.
SIMON HENRY GAGE, B.S., of Ithaca, N. Y., at Ithaca, N. Y., 1895 and 1906.
A. CLIFFORD MERCER, M.D., F.R.M.S., of Syracuse, N. Y., at Pittsburg, Pa., 1896.
A. M. BLEILE, M.D., of Columbus, Ohio, at New York City, 1900.
C. H. EIGENMANN, Ph.D., of Bloomington, Ind., at Denver, Colo., 1901.
CHARLES E. BESSEY, LL.D., of Lincoln, Neb., at Pittsburg, Pa., 1902.
E. A. BIRGE, LL.D., of Madison, Wis., at Winona Lake, Ind., 1903.
HENRY B. WARD, A.M., Ph.D., of Urbana, Ill., at Sandusky, Ohio, 1905.
HERBERT OSBORN, M.S., of Columbus, Ohio, at Minneapolis, Minn., 1910.
A. E. HERTZLER, M.D., of Kansas City, Mo., at Washington, D. C., 1911.
F. D. HEALD, Ph.D., of Philadelphia, Pa., at Cleveland, Ohio, 1912.
F. CREIGHTON WELLMAN, of New Orleans, La, at Atlanta, Ga., 1913.
CHARLES BROOKOVER, PH. D. of Little Rock, Ark., at Philadelphia, Pa., 1914.

The Society does not hold itself responsible for the opinions expressed by members in its published *Transactions* unless endorsed by special vote.

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Installments)
Vol. XXXIV APRIL, 1915 No. 2

THE DEVELOPMENT OF BOTANICAL MICROTECHNIQUE

By Gilbert Morgan Smith

Introduction

Development of our knowledge of cell structure has been correlated in a large measure with the development of methods of microtechnique, and it is a significant fact that Hooke did not discover the cell until he prepared sections of plant tissues. We are sometimes apt to think of the early investigators as having the same advantages we enjoy, but, in order to properly interpretate the relative value of any early botanical discovery, it is necessary to remember the means available to the worker of that time. In the present article the development of methods of making microscopical preparations will be treated from the botanical standpoint. The history of the microscope, and the development of culture methods will not be considered.

Advances made in microtechnique in Botany and Zoology are closely related. Discoveries made by zoologists in the art of making preparations were adopted by the botanist, while botanical methods were utilized by the zoologist. The microscopists, who were neither botanists nor zoologists, especially those under the influence of the London Society of Microscopists, have also played a very appreciable part in the development of the methods of making microscopical preparations. Probably for their development of microtechnique, rather than for their microscopical discoveries, are we indebted to the greatest extent to the microscopists.

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In tracing the development of animal microtechnique Apáthy has divided the time into three grand periods as follows:

1. The rule of the Dry Preparation, which lasted from the discovery of the microscope to the end of the 30's.
2. The rule of the compressorium and the razor, 1840-1880.
3. The rule of the microtome, 1880 to the present.

Perhaps a better method would be the discussion of botanical microtechnique under the following captions:

1. The methods of the early microscopists (from the time of Hooke's discovery of the cell to 1800).
2. The technique of the English microscopists (1800-1875).
3. The methods of the German botanists (1800-1875).
4. The development of modern methods of microtechnique (1875 to the present).

The Methods of the Early Microscopists

The microscopical laboratory, in the modern sense, did not exist in the 17th and 18th centuries. There are few illustrations of microscopists' work-rooms comparable with those that the chemist, physicist or pharmacist can show us. Ledermuller shows the way that a microscopist of that time fitted up a room for the purpose of showing his friends "some of the wonders of nature" by means of Cuff's solar microscope. An even better illustration of a laboratory is found in the head piece in each volume of Joblot's work (Fig. 1). This is probably an allegorical picture rather than an actual representation, but the microscope before the window, the hand microscope which is being used, the twigs in the vases and various objects on the table suggest the modern laboratory. The conspicuous position of the globe, the telescope and other instruments in the floor, however, do not fit into our present-day concepts but show the catholic taste of the early investigator.

Up to the beginning of the 19th century the microscope was a toy rather than an instrument of scientific research. Nelson mentions Pepys paying £10 for a microscope in 1664 and thinking it "a great price for a curious bauble." The attitude toward microscopy is also shown in the allegorical frontispieces of Ledermuller and of Adams. When Wilson says "In viewing Objects, one ought to be careful not to hinder the light falling on them, by the Hat,

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Perruke, or any other Object," we can easily imagine the casual manner in which the gentleman of that time looked through his microscope. Another conception of the purpose of the microscope is that of Baker (1742) who says "And if I can hereby induce any to pass those leisure hours agreeably and usefully, in contemplating the Wonders of Creation, which would otherwise be spent in tiresome Idleness, or, perhaps, some fashionable and expensive Vice, I shall think these Sheets very happily bestowed."

The first microscopists had to make their own microscopes as well as their microscopical preparations and, considering the primitive character of these instruments, it is natural to find them thinking the improvement of the microscope more productive of results than the improvement of the method of making their preparations. During the 18th century almost all works on the microscope were written by microscope manufacturers; so that great emphasis is laid on description of the construction of the instruments. These "Micrographias" were frequently sold with the microscope and were therefore written for the benefit of those who desired to dabble in microscopy. There was little serious use of the microscope, Baker stating the general attitude in the following: "Many, even of those who have purchas'd Microscopes, are so little acquainted with their general and extensive Usefulness, and so much at a Loss for Objects to examine by them; that after diverting themselves and their Friends, some few Times, with what they find in the Sliders bought with them, or two or three more common Things, the Microscopes are laid aside as of little farther Value. . . . ."

Hooke's microscope, as described in the "Micrographia," possessed no stage, the objects being mounted on a point attached to a pedestal at the base. Since Hooke prepared sections when he discovered the cell the following extract of his description is of interest. "I took a good clear piece of cork, and with a pen-knife sharpened as keen as a razor, I cut a piece of it off, and thereby left its surface smooth; then examining it very diligently with the microscope, ... but that possibly, if I could use some further diligence, ... I, with the same pen-knife, cut off from the former smooth surface an exceedingly thin piece of it: and placing it on a black object-plate, because it was itself a white body, and casting the light

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on it with a deep plano-convex glass I could exceedingly plainly see . . . ." A few years later in the Cutlerian Lectures he described a method of fastening "Muscovy Glass" to the bottom of the tube of the microscope in place of the "common Pedestal hitherto made use of in Microscopes." Another method used by Hooke is quite noteworthy, since it was used but little in the century following, and not until about 1820 did the process come into general use. He says: "But there are other substances which none of these ways I have yet mentioned will examine, and those are such parts of animal or vegetable bodies as . . . the Pulps, Piths, Woods, Barks, Leaves, Flowers, etc., of Vegetables . . . but if the same be put into a liquor, as water or very clear Oyl, you may clearly see such a fabrik as is truly very admirable . . . ."

Leeuwenhoek frequently made a microscope for an object that he wished to view and since these objects were generally fixed to a point on the microscope we may consider them as a sort of permanent microscopical preparation. Aside from the allegorical figure holding one of these microscopes in the frontispiece to the "Arcana Naturae" he has left no figures, while descriptions of his microscopes are known only from other writers. Upon his death a cabinet, containing several of these microscopes with their mounts, was left to the Royal Society of London and described by the vice-president, Martin Folks, and by Henry Baker (1753), before they were stolen from the Royal Society. The only original Leeuwenhoek microscope known to be in existence today is in the Utrecht cabinet, the Royal Microscope Society of London having a modern reproduction of the Utrecht microscope. In Folks' description we find; "Mr. Leeuwenhoek, fix'd his Objects, if they were solid to this Silver Point with Glew; and when they were Fluid, or of such a Nature as not to be commodiously view'd unless spread on Glass, he first fitted a little Plate of Talk*, or exceedingly thin-blown Glass, which he afterwards glewed to the needle, in the same manner as his other Objects." The figure of Leeuwenhoek's microscope as given by Ledermuller (Fig. 2) is particularly instructive since it shows the fine needle-like point on which the objects were mounted.

*The old usage of the word talc or "talk" is misleading since it refers to mica and not to the magnesium silicate called talc today.

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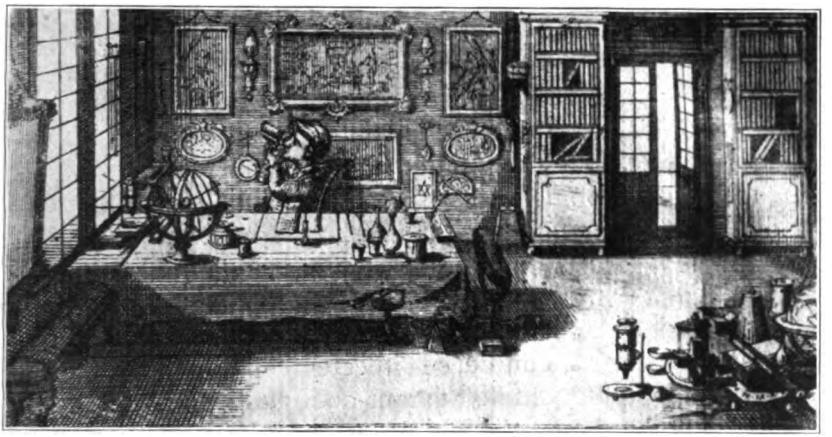

Fig. 1.—Allegorical representation of an eighteenth century microscopist's laboratory. (Joblot, 1754).

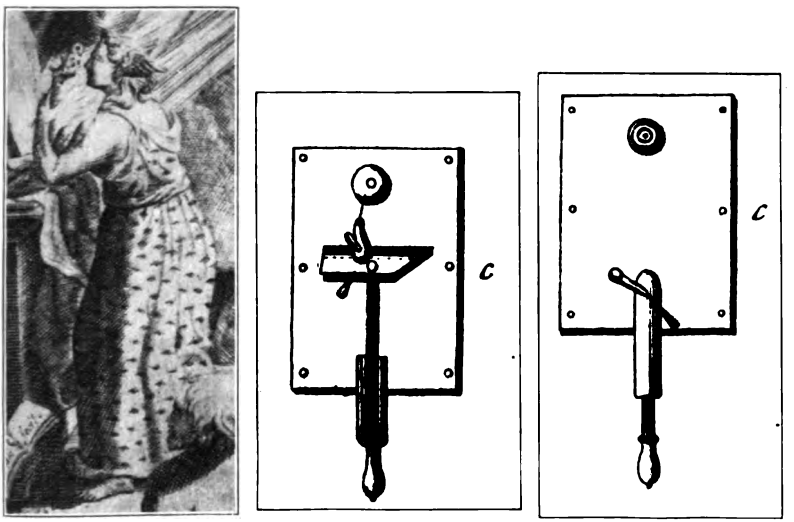

Fig. 2.—A. Leeuwenhoek's microscope (Ledermüller, 1768). B. An allegorical figure showing method of using this microscope. (Leeuwenhoek, 1722).

PLATE I

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In his study of blood corpuscles he first made use of small tubes for examining liquids. This method of exmaining material in capillary tubes became obsolete about 1800 but during the 18th century small glass tubes were a part of the equipment of all microscopes and Baker, Martin, and Adams all figure them. Leeuwenhoek's method of preparing these tubes was as follows; (1674) "I did myself prepare divers sorts of very slender hollow Glass pipes, of which some were not thicker than a Mans-hair; . . . . This pipe with the blood in it, I lay upon a piece of white paper, and with my nail break a little piece from it, and set it to the pin of my microscope, having first a little wetted the pin with my spitle, or a little turpentine, to make the pipe stick to it; or else I take the whole Glass-pipe and with my hand hold it before the microscope." This use of glass tubes for mounting material was almost exclusively confined to his zoological studies, and he later made the tubes larger and larger even describing one as large as a finger. (1702).

The founders of plant anatomy, Grew and Malpighi, have left but little record of their working methods. Malpighi says nothing at all about his methods, while all Grew has to say is; "to do all this by several ways of section, oblique, perpendicular, and transverse; all three being requisite, if not to observe, yet the better to Comprehend, some things. And it will be convenient Sometimes to Break, Tear, or otherwise Divide, without a Section. Together with the Knife it will be necessary to joyn the Microscope; and to examine all the Parts."

In the beginning of the 18th century we find object carriers or slides, ("sliders" as they were called at that time) coming into general use. About the earliest record of a slider that we have is the figure of one in position for use in the microscope of Philip Bon-

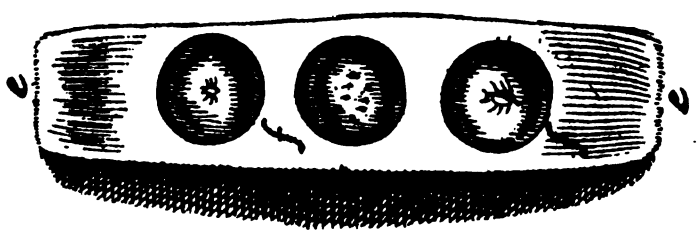

Fig. 3.—An early form of the ivory "slider". (Wilson, 1702).

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nani in 1698. Four years later in the Philosophical Transactions, Wilson shows one of these "sliders" (Fig. 3) describing it as follows: "EE a flat *piece of Ivory*, whereof there are 8 belonging to this set of Microscopes, (tho any one who has a mind to keep a Register of Objects may have as many of them as he pleases) in each of *which* there are 3 holes fff, wherein 3 or more Objects are placed between two thin Glasses, or Talks, when to be used with the greater Magnifyers." In this connection it should be borne in mind that material mounted in these sliders was always mounted dry. Hartzoeker (Fig. 4) used a hinged brass frame, in which the material was held between two pieces of mica, a process which was used but little. About the middle of the century glass "sliders" made their

Fig. 4.—Hartzoeker's frame for holding microscopic objects. (Harting, 1859).

appearance; Martin describing "a long piece of Glass, for moving the Object this way and that." The glass slide was not used for permanent preparations Adams (1747), Hill, and "Medicus" mentioning only temporary mounts with the glass "slider."

In 1742 Henry Baker devoted a chapter of seven pages to the subject "of preparing and applying objects." The need of preparation is seen in his statement that "Most Objects require some Management, in order to bring them properly before the Glass." The first method described, that of dry mounting in the "slider," is recommended for use wherever possible. Small concave glasses, quite similar to the watch crystals now in general use, are suggested for examining fluids containing organisms. In mounting these the material is to be taken up by means of a brush, which is figured among the microscopical accessories (Fig. 5 A). Baker also suggests the use of slips of glass, the same size as sliders, so that objects could be placed on them for examination; the interesting feature being the recommending glasses of different colors, giving as his reason; "many Objects being much more distinguishable when placed on one Color than on another." "Opake" objects are to be placed on small slips of colored cardboard, about half an inch in

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length and a tenth of an inch in width, and then fastened to the cardboard with mucilage. For preserving these preparations Baker

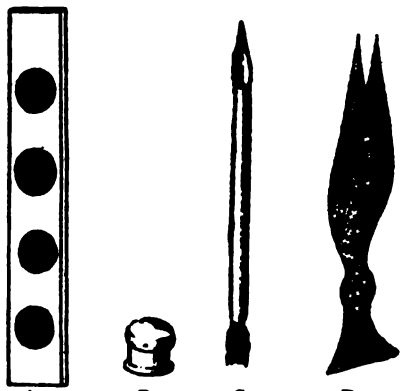

Fig. 5.—Apparatus for making microscopical preparations. A. Ivory Slider. B. Box of talcs. C. Camel's hair brush. D. Brass nippers. (Baker, 1742).

devised a box fitted up with compartments (Fig. 6), this being the first record that we have of boxes for keeping preparations.

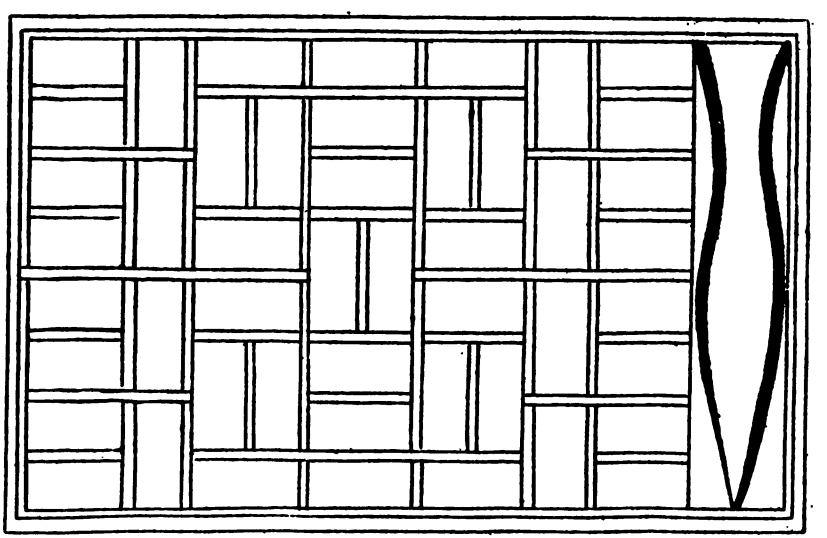

Fig. 6.—Case for preserving microscopical preparations. (Baker, 1742).

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Pollen grains are among the microscopic objects recommended by Baker and in Chapter 22 is found the method of making preparations of "Farina" (the old name for pollen grains). "Gather your *Farina* in the midst of a Sunshiny dry Day, when the Dew is off; be careful not to squeeze or press it, but shake or else gently brush it off with a soft Hair pencil upon a piece of clean white Paper. Then take a single Talc or Isinglass between your nippers, and breathing on it, apply it instantly to the *Farina,* which the moisture of your Breath will make adhere to it. If too great a quantity of Powder seems sticking to your Isinglass, gently blow off a little; if there be not enough breath on it again, and touch the *Farina* with it as before. Then put your Glass into the Hole of a Slider, and apply it to the *Microscope* to see if the little Grains are spread according to your liking, and when you find they are, cover them cautiously with another Talc, which fasten down with brass Wire, but let not the Glasses press hard upon the *Farina,* for that will destroy its true Figure, and represent it different from what it is."

Two further contributions to microtechnique which appeared in this century are in use to the present day. Ledermuller devised the use of dipping rods for removing material from a liquid (Fig. 7); while Benjamin Martin gives the first hint of maceration when he says; "If *Leaves* are steep'd in Water for Maceration, the Pellicle or thin Skin of both Sides will easily peel off, which laid on a glass and view'd with the Light reflected thro them, will discover a most delicate Texture. . . . ."

Historians emphasize the barrenness of the 18th century, as compared with the 17th, in the development of the microscope. With one notable exception, this is also true for microtechnique. The great originality which John Hill showed in the manipulation of the material described in his work entitled "The construction of timber, explained by the microscope" has not been given due credit. In his biography of John Hill, T. G. Hill has entirely failed to call attention to one of the most important features of John Hill's work, namely, the superiority of the microtechnique which he employed. Methods were used which had not been employed up to that time and which did not come into general use until fifty years later, and then as rediscoveries by others.

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Hill did not rely on any one method but studied the structure of stems in many different ways. He used a more elaborate method of maceration than Martin, and was the first to use maceration in the study of wood, sinking a loose wicker basket containing the sticks he wished to study into a stream until the tissues were well

Fig. 7.—Ledermüller's method for removing material from an aquarium. (1768).

softened. This identical method which was rediscovered by the younger Moldenhawer in 1812 is considered by Sachs as one of the great steps in the progress of phytotomy. Hill also makes the first mention of a method of preservation of material for further study. The practice of dropping the macerated pieces of wood into a solution of alum and then transfering them to spirits of wine, after

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thoroughly drying, resembles in a very crude manner our modern method of fixing and hardening. The reason for this is seen in his statement that "Nothing but spirit of wine can preserve these tender bodies, and, till I found this method of hardening them first, the liquor often destroyed them."

Holzner thinks that Sarrabat or Reichel should have the credit for being the first to use staining methods since they put sticks into colored liquids and then noted the rise of the color. Apáthy has raised the question as to whether this work should be regarded as at all comparable to our modern methods of staining microscopical preparations. Judging by the excerpts cited from these articles by Holzner they were macroscopical studies only, and it is very probable that Hill was not aware of them, or the work of Bonnet.

Hill is undoubtedly the first to have used staining as an aid in the study of microscopical anatomy of plants. He prepared an alcoholic tincture ofcochineal, in which, after it had been filtered, he placed the stems of plants for a while, discarding that portion which had been immersed in the fluid when he made his sections. Another method of staining used was even more advanced since it involved a mordanting of the tissues before developing the color. A solution of sugar of lead was prepared, filtered, and put in a tea-cup and the sticks to be studied were allowed to remain in this fluid for two days. An essential part of this operation was the "whelming" of the tea-cup with a wine glass to prevent the drying of the material. While the tissue was soaking in the lead solution he prepared a solution of quick lime and orpiment in water and then transferred the material from the tea-cup to the second solution for two days. When the sticks were first placed in the second solution they were colorless, but in a short time they became deep brown. By means of this staining he was able to demonstrate the existence of structures invinsible in the uncolored material. A third method, which was an injection rather than a staining process, was the careful drying of the wood and then boiling it in green sealing wax. By this procedure the vessels became thoroughly impregnated with the green sealing wax and the "split pieces resemble striped satins, in a way scarce to be credited."

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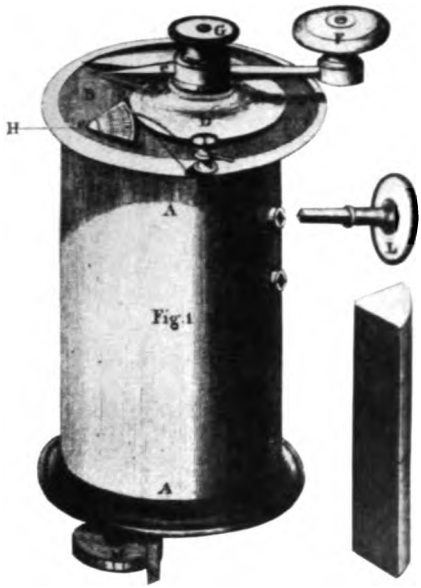

Fig. 8.—Cumming's microtome of 1770. (Jour. Roy. Micr. Soc., 1910).

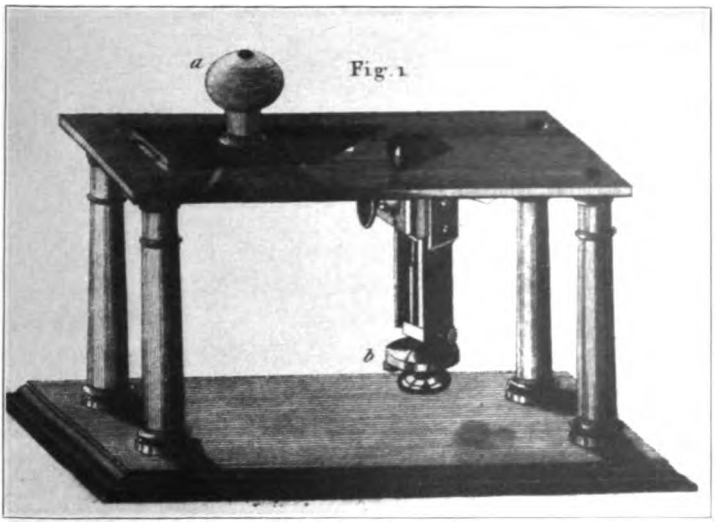

Fig. 9.—Adam's cutting engine. (1798).

PLATE II

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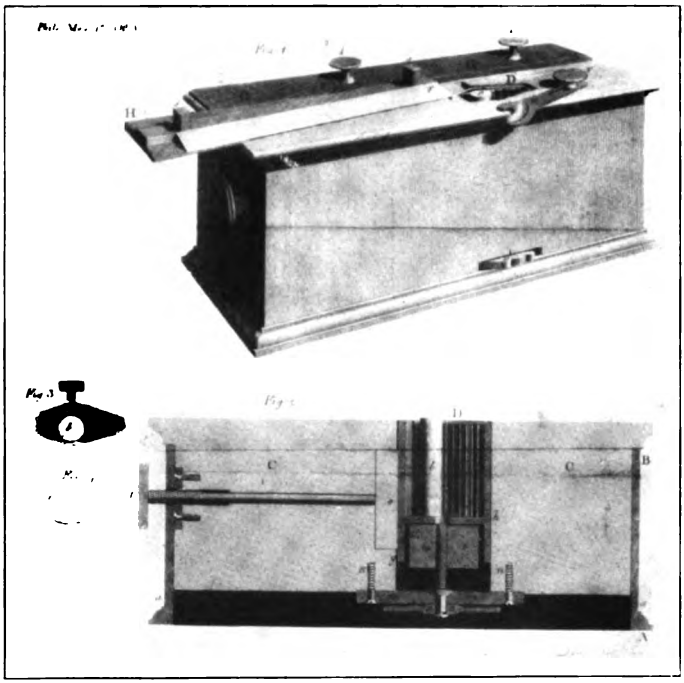

Fig. 10.—Custace's cutting engine. (Thornton, 1799).

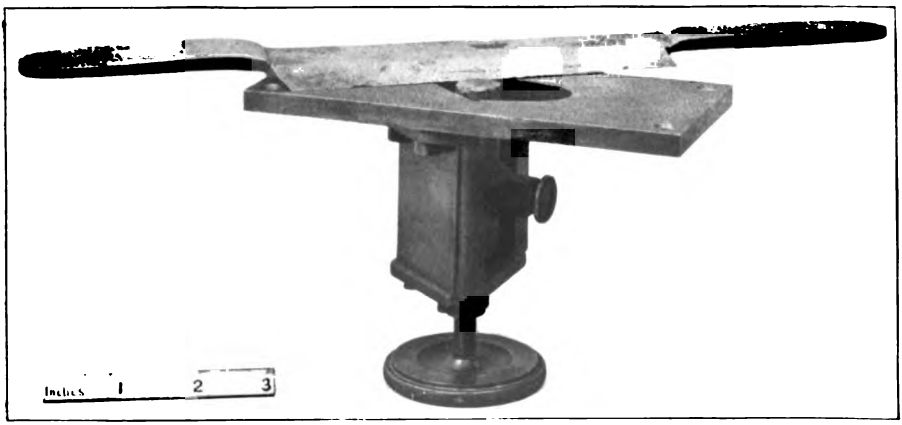

Fig. 12.—Pritchard's microtome (1835).

Plate III

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Other methods of study used by Hill include the placing of tissues of pine in spirits of wine until the resins were dissolved out and the cells rendered more visible. Another, the placing of tissues in spirits of turpentine until the contents became clear. Both of these methods, although extremely valuable in the study of the anatomy of plants were not used until a much later period. In this study a slide of ground glass was used in addition to the ivory "slider", but both of them were used in the holder devised for the purpose instead of placing them directly on the stage of the microscope. As to his methods of making mounts with the glass he says: "it is to be examined, if fresh, in water; if preserved in some of the spirits in which it is kept; being laid in a little cistern hollowed in a slip of ground glass." This form of slide devised by Hill is generally regarded as being a comparatively modern invention. These methods of study developed by Hill received but little notice, Adams seemingly being one of the few to recognize their value as is seen when we read; "it were to be wished a satisfactory account could here be given of all the preparations which are requisite to fit for the microscope the objects of the vegetable kingdom. Dr. Hill is the only writer who has handled this subject."

The sections that Hill used were cut out on a microtome. Queckett states that the first cutting machine (microtome in our sense) was made by Adams about 1770. The instrument that Hill used (Fig. 8) was one invented by Cummings and was probably well known at the time since after making two or three Cummings turned their manufacture over to Ramsden who supplied them to those desiring "cutting engines." The body of the instrument (AA) was made of ivory, while the top was of bell metal. The spiral-edged cutter was so arranged that the difference between the longest and shortest radii was greater than the thickness of the largest piece of wood that the instrument would hold. The handle (F) was used to revolve the spiral cutting blade and after each revolution of the blade the material being sectioned (H) was raised the desired height by means of a screw (M), each division on the head of the screw corresponding to an elevation of the material 1-1000 of an inch. Hill was troubled by the sections of wood curling as they were cut, so a fine spring was used to keep them flat. After

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cutting, the sections were transferred to spirits of wine. Those interested in a more complete description than is given here, and to whom the work of Hill is inaccessible, will find a reprint of Hill's description and plate in the Journal of the Royal Society for 1910.

Thanks to the love of the early microscopists for careful descriptions of the minutiae of manipulation we have a good account of the state of microtechnique at the close of the 18th century given by Adams. In these "Essays on the Microscope" a description is given of all the microscopes in use at that time, and, what is of greater interest from our point of view, he carefully figures and describes all the accessory apparatus which accompanied those microscopes. All were supplied with ivory sliders which fitted into slider holders made of brass, while the objects were mounted dry between pieces of talc and held in the holes of the sliders by brass wires (Fig. 5 A). The sliders as supplied usually contained objects ready for examination, but an empty slider or so was sent along, as well as a box of extra talcs (Fig. 5 B), so that the owner of the microscope could make preparations if he desired. Other accessories needed in the preparation of objects were camel's hair brushes (Fig. 5 C) and brass nippers for adjusting the brass rings that held the talcs in place (Fig. 5 D). Approximately half a century separate the first edition of Baker's "The microscope made easy" and the last edition of Adams' "Essays" yet little improvement is noted in the accessory apparatus figured, the apparent advancement shown in a comparison of the figures of Adams and Baker being due rather to better draughtmanship.

Adams described an improved instrument "for cutting thin transverse sections of wood." This "cutting engine" (Fig 9) consisted of a wooden base which supported four brass pillars that in turn bore a flat plate of brass, in the center of which was a triangular hole. The piece being sectioned was placed in a triangular trough on the under side of the brass plate, and fastened by a brass screw. A diagonal knife blade, greatly resembling the blade of a plane, did the cutting. This was moved back and forth by a handle, its course being governed by two grooves in the top of the brass plate. The amount that the block was raised was governed by

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a micrometer screw. In cutting, only fresh material or well soaked material was used and this was kept flooded with alcohol to prevent the curling of the sections. That other microtomes besides those of Cummings, Adams, and Custace were known may be judged by the foot-note of Kanmacher (the editor of the second edition of Adams' "Essays"). "Many other kinds of cutting engines have been constructed, but specimens from them have not yet appeared with the perfection which is requisite to this sort of objects; whether it lies in the preparations of the woods, or engine, I do not take on me to determine."

In this work also appears the first record of the dealer in microscopical preparations, the last chapter containing a list of "vegetable cuttings" which Custace supplied to those interested in microscopy. His sections were all prepared by means of the microtome that Thornton has described. Custace was quite famous for the sections that he made, his preparations being supplied with all the high-grade microscopes of that time. Even as late as 1852 we find Queckett saying: "some of his (Custace) preparations have not been improved on to the present day." Custace, who was a "common carpenter of Ipswich" kept his methods of making preparations secret during his life, refusing an offer of £50 from Thornton for a description of his methods. After his death all his effects were auctioned off and Thornton "fearful that a monopoly might be made of the art of preparing vegetable cuttings, as had been successfully done by Custace," bid in the two microtomes offered for sale. Thanks to the generosity of Thornton we have a description of these "cutting engines." The outer case of the cutting machine (Fig. 10) was made of brass, in the form of an oblong box, which was completely filled with a block of hard mahogany except for the holes necessary for the mechanism that held and raised the material being sectioned. The block was raised by a micrometer screw, which is not shown in the illustration, the screw being operated by an index wheel (O) at the side of the box. The large screw at the left of the microtome was used to clamp the block in the brass "Holdfast" (D). Especial attention was paid to the designing of the mechanical means for guiding the knife, and it may be due to this that the machine cut such good sections. The knife blade was

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set diagonally on a bed (H) which slid back and forth along a steel rod (GB). Another device for securing rigidity was the placing of a spur (u) on the top of the microtome. This spur held the material in its crotch and was prevented from giving by a screw (v).

In concluding the discussion of microtechnique before 1800 the following items might be of interest. They are taken from the advertisement of W. & S. Jones and appeared in Adams' "Essays."

	£	s	d
Common Microscopes from 2 s. 6 d to.....	1	1	0
Compound Microscopes from 2/12/2 to.....	5	5	0
New Improved ditto with most complete apparatus....	10	10	0
Cutting Engine for slices of vegetable objects.....	3	3	0
Ivory Sliders, for transparent objects (per doz.).....	0	12	0
Custace fine vegetable cuttings in large ivory sliders, from a set of six sliders to four dozen (per doz.)....	1	10	0

The Technique of the English Microscopists (1800-1875)

With the beginning of the 19th century we find botanical microtechnique developing along two distinct lines. With the rise of the German phytotomists there was a high degree of specialization along the line of microchemistry while comparatively little attention was paid to refinements in methods of making preparations. In England, on the other hand, with the reawakening of interest in the microscope, attention was largely confined to developing methods of making microscopic preparations. Non-scientific Englishmen, as well as the microscopists, were interested in the microscope and so we frequently find articles on the microscope, or on microscopic objects in the popular magazines. Examples of these popular articles on the microscope are those which appeared in the *Saturday Magazine* or the *Mirror*. Popular treatises on the microscope also appeared and through the influence of such works as those of Brewster or Pritchard (1847) there was a recruiting of a body of microscopists. With a few notable exceptions the English microscopist made no great contributions to botany, that were based on microscopical observation, but they were largely instrumental in advancing microtechnique. It has been through the refinements zoologists have made in the Eiglish microscopists' methods, and then in turn their adaptation by botanists that we have the botanical microtechnique of today. Indirectly also we are indebted to the microscopists

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for the great stimulus that their interest in the microscope, and their willingness to purchase it, gave toward the improvement of the instrument; hence the development of the microscope up to comparatively recently has been due almost wholly to demands of the microscopist for a better instrument.

I have previously mentioned that the early microscopists did not work in regular laboratories. A great majority of the men in England who were interested in microscopy in the period under discussion were not connected with an institution where they would have regular work-rooms, so worked in their homes. Consequently writers on microscopy give directions for the best conditions for work. Goring and Pritchard recommend a separate room, which they call an observatory, that is always to be kept locked when not in use. The difficulties with which these workers were beset is brought out in the following: "Have the fear of the cat before your eyes, and also those busy, intermeddling, officious, housewives, who, under pretense of dusting, cleaning, and setting to rights, will subvert and revolutionize the whole economy of your observatory, and perhaps throw half your tackle behind the fire."

The use of the ivory and wooden slider continued until the first or second decades of the 19th century, though they did not become entirely obsolete until about 1860, some of the inferior microscopes at that time being furnished with them. With the general introduction of the glass slide the methods devised for the ivory slider were adapted to the glass slider with a few changes. This modified method is described by Gould who in making permanent preparations took two pieces of glass, of the same size, and then pasted on one a piece of paper containing one or two holes. After the mucilage was dry the material was placed in the holes in the paper, the other glass pasted on the paper, and the whole held together until thoroughly dry. That this process became quite general is seen in its description by Griffith, von Mohl, Queckett, Harting, and others.

The publications appearing in the years 1830-1835 mark the foundation of the English microscopist's methods. It is impossible to state who first substituted the glass slide with a cover of talc for the ivory or wooden slider in making preparations, but that it was a well

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known method by 1830 may be judged by its use by Pritchard (1832) Varley, Solly and Holland. Pritchard's revolutionary method of mounting dissections in a thick solution of gum and isinglass and then covering them with a thin plate of talc was described in 1832 but did not come into general use since it was so soon superseded by mounting in Canada balsam. Credit for the introduction of Canada Balsam is generally given to J. T. Cooper who suggested its use to New and Bond, professional microtechnicians of that time, but the publication of the method is due to Pritchard (1835). Balsam may have been used before this since Adams states the following about Swammerdam's methods of preparing insects for microscopical observation: "Sometimes he has examined with the greatest success, and made the most important discoveries in insects he had preserved in balsam, and kept for years together in that condition." I have been unable to find this description in the work of Swammerdam, but Adams may possibly have taken this from Boerhaave, an article which I have not personally consulted. However, it was through Pritchard's publication that Canada balsam became widely known as a mounting medium. The first mounts made differed considerably from those we now use since Canada balsam was taken in the natural state, instead of dissolving it in some solvent, and after melting a small piece on a slide the object was mounted before the balsam hardened. Judging by the space given to the description of the process in the older works on microscopy there must have been considerable difficulty in making the preparations and all sorts of mechanical contrivances were devised to hold the cover in place during the drying of the balsam, to melt the balsam, to remove air bubbles from in under the cover glass, etc.

The development of methods of mounting objects in liquids is of even greater significance. The earliest record I have been able to find is by Goring and is as follows: "I have neglected to describe a kind of slider which I use in my microscope; it is composed of a glass tube, flattened, and drawn out to the size of a common slider, and polished on one side; its use is to hold microscopic objects which will not keep in a dry state, such as pieces of finely injected membrane, petals of flowers, and the like; these little preparations are introduced into the slider, which is filled with spirits, and cov-

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ered at the end with a bit of bladder secured by a wax thread". This is the only record of this type of permanent preparation. In 1829 a short anonymous note stated that Holland had covered material, which showed the Brownian movement, with a talc and then hermetically sealed the preparation. Four years later Holland described the method of making these "ponds" by taking a piece of glass and enclosing a space on the glass with a cement composed of white lead and turpentine and then covering with a talc and sealing. Although devised to show the Brownian movement Holland stated that the "pond" could be used for tissues. Previous to the publication of the process by Holland, Varley had described essentially the same method for preserving "minute vegetable dissections" and called this type of preparation a "cell," the name by which they are universally known at the present day. White lead cells were also made as early as 1830 by Valentine, Solly mentioning preparations of Valentine's made at that date. It is possible that this type of preparation was known even earlier for in 1841 Daniel Cooper said that Dr. Cook recommended a mixture of salt and water as a mounting fluid "20 years ago" (i. e. about 1820). Pritchard's process (1832) is even more important for he is the first to have suggested mercury bichlorid as a mounting fluid. Daniel Cooper says that J. T. Cooper used salt and water with a little acetic acid for mounting vegetable tissues, although he does not give the date on which Cooper proposed this method.

Goadby is generally credited with being the founder of the methods of making moist preparations, but the citations just given show that methods of making moist preparations were well known before the work of Goadby and any credit due him is more for perfecting methods in use than for original discovery. He is best known for the mounting fluid that bears his name. That this process was recognized as being revolutionary is seen by the fact that the Society of Arts gave him a gold medal in 1841 and raised a private subscription of £500 to purchase his preparations for the Hunterian Collection of the Royal College of Surgeons (Goadby, 1852). The formula for the solution was first published by Daniel Cooper in 1841, but since there seems to have been considerable difficulty in making preparations in this way Goadby described his meth-

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ods and gave his formulae before the Section of Botany at the York meeting of the British Association in 1844. The Gannel process described by Cooper is unknown except for this single reference. It was quite similar to Goadby's method except that "superacetate of aluminia" was used.

The attitude of the British microscopists towards their discoveries was in marked contrast to that of investigators of other countries. There seems to have been a great freedom of oral interchange of ideas, and one man often devised a really epoch-making improvement in microtechnique but made no attempt to publish it, being content to communicate his discovery to his friends by word of mouth. Michael has explained how microscopists came to see so much of one another when he says that Bowerbank practically kept open house for microscopists before the formation of the Microscopical Society of London. After the formation of the Society in 1839 there was a formal meeting place for microscopists but the practice of holding "soirees" at various times favored the oral interchange of ideas. To mention only a few instances, we have Pritchard publishing Cooper's method of mounting in Canada balsam, Cooper publishing Goadby's formulæ, and Clarke the first method of clearing, a process which he may possibly not have invented.

Holland is the first to describe the deep cell that is made by applying successfully several layers of cement. The importance of the cement cell in microscopy at this time is seen in the number of

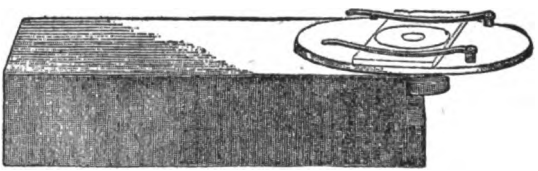

Fig. 11.—An early form of turn-table for making cement cells. (Carpenter, 1856).

cements described by Griffith and Henfry, Queckett, Carpenter, and Beale. The chief contributions to the subject of cements were Berkley's description of Thwaite's method of using gold size, and Reckitt's recommendation of black Japan on account of its quick drying properties. At first these cells were made by hand, but the invention

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of the turn-table by Shadbolt gave a quick mechanical means of making them. The first turn-table is figured by Queckett, but it was soon improved and the instrument we use today shows little advance over the form given by Carpenter in 1857 (Fig. 11).

Griffith gives the status of microtechnique in the early forties where he says that the use of ivory and wooden sliders has almost disappeared but he describes a method of dry mounting very similar to that of Gould. The method of mounting dry objects in balsam, and several methods of making moist preparations are also given. For the latter a syrup and gum mixture, dilute alcohol, water saturated with creosote, and Goadby's solution are described, the last named being given the preference. In mounting these preparations the sealing with some varnish, the use of white lead cells, and built-up cells are all described.

Goadby's solution proved altogether too strong for plant material and consequently a number of media were devised which had as their object the avoidance of plasmolysis. Thus Reckitt advised sealing the tissues in either pure water or a dilute solution of corrosive sublimate, while in 1849 Warrington recommended castor oil for certain fungi since he had found this such a good mounting medium for crystals a few years previous. The desmids have always been a favorite object for study with the microscopists and we therefore very naturally find several formulæ of mounting fluids for these delicate organisms; among them may be cited Thwaites' mixture of 1 part alcohol and 12 parts water with as much creosote as could be dissolved, a proportion which was later changed to 1 part alcohol to 16 parts of water when the process was described in Ralfs' work on the desmids. Ralfs medium for desmids consisted of a grain each of bay salt and alum dissolved in an ounce of water. Glycerine was first used by Warrington in 1849 for mounting microscopical preparations, but since glycerine alone caused too much plasmolysis Farrants proposed a jelly of equal parts of glycerine, gum arabic and water, while a formula essentially the same as the glycerine jelly used today was given by Lawrance in 1859. The idea of using gelatine as the foundation for the mounting fluid really belongs to Deane who first proposed a mixture of honey, water, alcohol, creosote, and gelatine.

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Although all of these methods were described before 1860, we must bear in mind that they were used only in special cases, Canada balsam being considered the prëeminent medium whenever possible. The column of questions and answers appearing in *Science Gossip* during the sixties contains many more references to Canada balsam than to any other method. A revolutionary step in the manipulation of Canada balsam was the dissolving of the balsam before mounting. This was first used by Griffith in 1843 but does not appear in the article on microtechnique by Griffith but as an editorial note of a few lines in the same volume that contains his description of microtechnique. The use of a solvent for the balsam did not come into general use in England, until more than a decade later about the only reference to it being those of Boys and Ralph.

Another great step in microtechnique appearing in the decade between 1850 and 1860 is the introduction of the process of clearing tissue before mounting them. In 1851 Clarke, in describing his treatment of certain animal tissues, stated that he put them in spirits of wine, then transferred them to turpentine and after they had become quite clear mounted in balsam. It is possible that he did not originate this method since Farrants remarked in 1857, incidental to a discussion of the use of glycerine, that he had cleared his material in turpentine before mounting in balsam since 1850, but makes no mention of Clarke's name. From the historical standpoint Clarke's publication of the process is important for it was through his description that it became known abroad and in the hands of the German zoologists of 1860-1870 developed into the methods of clearing that still persist. Evidently the value of this method was not recognized in England since the treatises of Queckett, Griffith and Henfry, Carpenter and Beale all fail to mention it.

The cutting engine as invented by Adams, Cummings, and Custace was used more or less in England between 1800 and 1870. After speaking about these 18th century microtomes Queckett goes on to say "in subsequent times other instruments have been contrived for the same purpose, some provided with knives that move circularly, others with knives fixed in a strong framework of metal, whilst, in not a few, the cutting is performed by a razor of the ordinary kind, or one ground perfectly flat." I have been able to find

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reference to five microtomes, other than the two described in the first edition of Queckett in 1848, that were made before 1850 but judging by the statement of Queckett many more were known. Michael says that before the foundation of the Microscopical Society of London George Jackson made "a very servicable cutting-machine for producing thin sections of wood." The firm of Charles Baker of London inform me by letter that they have been making microtomes since about 1840. In 1836 Bowerbank described a microtome that he had invented which was quite similar to the Adams instrument, the chief difference being that the cutting part of the instrument was a razor ground flat on one side. There are also two old microtomes in the Science Museum, South Kensington, London, that were made about 1835*, one bearing the name of Andrew Pritchard, while the other bears no maker's name but is of very similar design. Pritchard's microtome (Fig. 12) is described as follows in the Science Museum catalogue. "The apparatus is made to be screwed to the edge of a table and consists of a flat plate of brass with a well in it, in which a kind of piston moves up and down by a micrometer screw. The wood to be cut is fixed to the piston by a small screw, and as it is raised a knife drawn along the plane surface takes off thin sections. Should the piece of wood be too small to be placed in the triangular chamber, it must be glued to a block of convenient size." This is the first record of a microtome fixed to a table and is the forerunner of the hand microtome.

The instrument of Topping was patterned after the Pritchard microtome and was the best known microtome of the 50's, being figured and described by Queckett, Carpenter, and Beale. It is quite similar to the hand microtomes of the present day, resembling them more than the Pritchard microtome. Another microtome is one that is ascribed to Queckett by Harting and Apathy, although I find no direct evidence that Queckett invented this instrument. It consisted of a mahogany base (Fig. 13) that supported four brass pillars and a top plate. The "well" for holding the material was essentially the same as has been previously described for the Adams cutting engine.

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* I am under very great obligations to Dr. A. B. Bendle of the British Museum for his kindness in furnishing me information concerning these microtomes, and to Capt. H. G. Lyons for the photograph and description of the Pritchard microtome.

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The knife differs considerably from that of the Adams cutting engine in that it was placed diagonally on a brass frame, the whole sliding backwards and forwards along a guide rod on the top of the cutting engine.

The great interest in microscopy in the decade of 1850-1860 is evinced by the appearance of five treatises on the microscope and microscopic manipulation. With the appearance of these works, methods which had been but little known outside of a small circle of

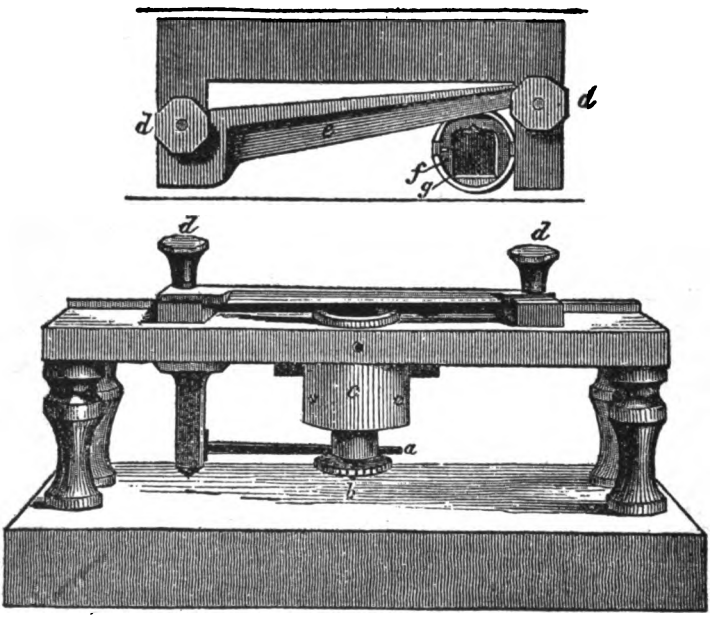

Fig. 13.—Queckett's (?) microtome. (Queckett, 1848).

microscopists became the property of the world and were taken up and perfected outside of England. The advances made in microtechnique by the introduction of the complicated paraffine method made the science one for the laboratory rather than for the home and although interest in microscopy continued in England the progress of the science took place in laboratories of the German zoologists. The most important contribution of the microscopists to microtechnique between 1860 and 1875 was the introduction of staining,

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particularly the staining of plant tissues, although the German botanists did not avail themselves of the staining methods of the English microscopists. Since the discussion of this subject will be taken up later no further mention will be made here.

The French microscopists were dominated to a large extent by the English school of microscopists and have played a relatively unimportant rôle in the development of microtechnique. Several of the English works on the microscope were translated into French, Lebour's Galerie Microscopique, for example, being the French edition of Pritchard's Microscopic Cabinet. In the works written by the French themselves English methods were drawn on to a much greater extent than the German, Chevalier and Dujardin both showing quite strongly the influence of the English microscopists. Chevalier deserves credit for being one of the first to substitute thin glass covers for the talc in making permanent preparations.

Botanical Microtechnique in Germany 1800-1875

As far as the art of making microscopical preparations is concerned the technique of the German botanists was far behind that which the English microscopists used in making preparations of botanical material during the period described above. This was due to the German botanist's belief in the utmost simplicity. To emphasize the necessity of great dexterity with a few instruments, von Mohl, Harting, and Behrens all quote Benjamin Franklin's adage that "a naturalist must saw with an auger and bore with a saw". Such great manual dexterity was developed in cutting free-hand sections, that mechanical instruments were thought to be only for those who could not make good free-hand sections. Thus in his review of the description of the Oschatz microtome, von Mohl held that the Oschatz microtome was of real value only when one wished to prepare large sections for a microscopic cabinet, and that for scientific investigation the microtome was highly superfluous.

In Germany as in England the dry mount was used exclusively at first. With the rise of the Phytotomists there is some evidence of use of water mounts, although it is not clear whether the material used by Link and Kaulfuss was merely examined in a drop of water or whether this drop was covered as we do today. The water

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mount was quite generally used by the later Phytotomists; Meyen in his directions for manipulating the microscope (1830) telling us to use a glass slide and a drop of water and then cover it. No permanent preparations were made in Germany before 1840, all sections made with the razor being examined in temporary water mounts.

Kaiser (1877) states that Germany did not take up microscopy until about 1839 and that Moser was the first to bring about a development of microtechnique, while Behrens thinks that von Mohl was largely instrumental in creating this interest in microtechnique. The chief contributions to the technique of making permanent preparations are those of von Mohl, Oschatz, Schact, and Harting. English methods were not drawn on until about 1850 although the papers of Griffith and Varley had been translated into German. The year of the translation of Griffith's paper marks the appearance of Oschatz' methods of making permanent preparations. Oschatz being a microscopist, discussed both animal and plant tissues. For the latter water alone was not recommended but either a concentrated sugar solution, or a sugar solution containing a little acetic acid was recommended. When the young plant tissues were too opaque, Oschatz found that they could be cleared to a considerable extent by placing them in acteic acid before mounting. The following year Moleschott described Harting's process of mounting plant material in a concentrated solution of iron-free calcium chlorid so that the technique of making permanent preparations received a great impetus at this time. Harting took a slide and pasted a strip of paper on each end and then mounted the material in a drop of the calcium chlorid solution in the center of the slide. This was covered with another slide of the same size and the two fastened together with paste on the strip of paper. There was no necessity for sealing this preparation since the hygroscopic nature of the calcium chlorid prevented evaporation. An interesting side light showing what was considered essential in the study of the cell at that time is found in Moleschott's comment on the availability of the method. He says that apart from the swelling and dissolution of the starch grains, the dissolution of the nucleus in a few months, and the shrinkage of the surrounding membrane in many cases (von Mohl's

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primordial utricle), the method of mounting in calcium chlorid solution is a very good one. Immediately following Moleschott's description there is a comparison by von Mohl of Oschatz' and Harting's methods in which he thinks Harting's process is the superior and he bemoans the fact that it had not been discovered earlier. The chief objection to mounting in a sugar solution was the inability to seal the preparation properly, von Mohl's preparations usually not lasting over a year. On the other hand Münter thought Oschatz' method the best. The "Mikrographie" of von Mohl is one of the first collections of its kind in Germany and gives a good idea of the methods in use at that time. It may be well to note that the first edition of Queckett, which appeared at approximately the same date, devotes about half the pages to methods of microtechnique, while in the 277 pages of von Mohl's work only 27 pages are given to the subject, thus showing the difference between what was considered essential in microscopy in England and in Germany. Von Mohl thought that most organic bodies should be studied in water mounts since balsam rendered them too transparent. A method of mounting dry preparations, essentially like that of Gould, was described. The technique of Oschatz, Griffith, Thwaites and Reckitt was described but scarcely any attention paid to the making of cells, all preparations containing fluids being hermetically sealed by some varnish after the cover had been placed in position. The calcium chlorid method of Harting was recommended whenever possible, but the swelling of the starch grains and the shrinking of the primordial utricle prevented its universal use. Owing to his strong advocacy of calcium chlorid von Mohl is frequently credited with being the originator of this method.

The formation of the "Verein für Mikroskopie zu Giessen" in 1856 helped standardize methods. This society adopted, after considerable experimentation, a uniform object carrier for all those members who wished to exchange preparations. They rejected the English size of 1 by 3 inches, a form that came into use in that country soon after the foundation of the Microscopical Society of Lon-

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don, and used one 33 by 28 mm. instead.* In the by-laws of the society reported by Leuckart and Welcker the following abbreviations show the mounting materials most generally used in Germany in 1856; Al. alcohol, CB Canada balsam, CC calcium chloride, Gi. gum arabic, Gl. glycerine, Lc. liq. conservatoire (Paccini's fluid), WG water glass, Z. sugar, O. dry mount.

The German botanists were familiar with the publications of the English microscopists which were appearing about this time and so we find a gradual abandonment of the calcium chlorid and the concentrated sugar solutions as the exclusive mounting media. This recognition of the English microscopists' methods seems to have been due to Welcker's publication, judging by the statement of von Mohl in 1857. Unfortunately I have been unable to consult the original paper of Welcker. Von Mohl here gives the preference to glycerine over calcium chlorid as a mounting medium. Another publication which greatly influenced botanical microtechnique was that of Schact, which appeared in 1851. This is the first work devoted exclusively to plant histology. Comparatively little attention is given to making permanent preparations, only three methods being mentioned, namely, the use of calcium chlorid, sweet oil, and Canada balsam, but because of the minute directions for the anatomical study of different plants the book was of great value. This work also illustrates the difference between the microscopical methods of the English and the Germans. In the case of the English the preparation was the main thing, while with the Germans the preparation was only a means to an end.

*The proper size for the object carrier was a subject of considerable controversy in Germany. The following are some of the sizes recommended.

78 x 26 mm. (London format, 1840)
70 x 22 mm. (von Mohl, 1840)
30 x 40 mm. Diameter circular plates. (von Mohl, 1840)
2/3 x 2/3 in. (Oschatz, 1851)
33 x 28 mm. (Giessen format, 1856)
55 x 26 mm. (Frankfort format, cited by Leuckart and Welcker in 1857)
70 x 20 mm. (Gerlach, cited by Leuckert and Welcker in 1857)
37 x 22 mm. (von Mohl, 1857)
43 x 28 mm. (von Mohl, 1857)
48 x 28 mm. (New Giessen format, date of introduction unknown)
65 x 25 mm. (Vienna format)

Perhaps the new Giessen format of 48 x 28 mm. has been the most used in Germany up to the last decade, but at present the English format is in almost universal use. For other sizes less frequently used in modern times see Behrens.

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If the German botanist was not fully abreast of developments in the technique of making preparations, he more than compensated for this in the forwarding of botanical microchemistry. By 1800 unorganized studies are found in which nitric acid, hydrochloric acid, the solvent action of alcohol, and the like were used in an attempt to discover the nature of the cell contents. Perhaps the researches of the French botanist, Girod-Chantrans may be taken as typical of this blind groping toward a microchemical study of the plant tissues. One of the earliest definite microchemical reactions is the discovery by Link in 1807 in which he used iron sulphate for determining tannic acid in leaves. In the same article Link relied on warm water, sulphuric and nitric acids as a test for starch. The macrochemical reaction of starch and iodine (discovered independently by Stromeyer, and Colin and de Claubry), was used microchemically by Raspail in 1825. Four years later Raspail found that sulphuric acid gives a purple coloration to albumen in the presence of sugar. Raspail used this reaction either for the determination of albumen or sugar, when it was present in large quantities, as in pollen grains. Schleiden in 1838 and von Mohl in 1840 showed that after treating the cell wall with sulphuric acid, iodine caused a blue coloration of the cellulose. Ten years later, in 1850, this method was largely supplanted by Schultze's zinc chloride solution. In the same year Millon devised the test for proteins that bears his name. Thus we find that by 1850 the microchemical determination of the constituents of the plant cell was in a fairly satisfactory state. Although Schleiden gave a list of chemicals for the study of the cell in 1842, the first serviceable collection of microchemical methods is that of Schact in 1852, in which the means of recognizing many different plant products is given. Thus cellulose and xylogen can be differentiated by the reagents given, protein compounds recognized by their behavior towards iodine, nitric acid (a reaction which was pointed out by Glauber in 1686), hydrochloric acid, and Raspail's test. Starch is recognized by iodine, gums and dextrines by digestion and the formation of a flocculent precipitate in alcohol. No very sure method is given for sugar or fats, Raspail's test showing sugar when present in abundance, while fats are determined by their high refractive power and disappearance under the microscope

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in the presence of alkalis. Other important advances were the application of Troemmer's reagent to microchemical analysis by Sachs in 1859 and the use of alcanna tincture by Mueller for the determination of fats. The growth of microchemistry may be seen by comparing Poulson, Behrens, Zimmermann, and Richter.

The work of the Hofmeisterian epoch which led to the foundation of our knowledge of the alternation of generations was carried out almost wholly on fresh material and with free-hand sections. Botanical study in Germany between 1840 and 1875 was dominated by von Mohl, Schleiden, Hofmeister and Naegeli, all of whom favored the use of as simple a technique as possible, their mastery of free-hand use of the razor being traditional even to the present day. It may be that the great skill which they possessed with the razor made them loath to use the microtome and although Unger and von Mohl mention the microtome they did not recommend it but gave the impression that it was an instrument for the dilletante rather than for the serious worker. Even today this idea lingers in certain quarters and is expressed in the saying that "a steady hand is the best microtome." In certain cases special methods of holding the material were recommended, von Mohl using pieces of pith for holding thin leaves and the like, whereas Unger used cork. Schleiden in 1842 described a method of fastening material to the thumb-nail with saliva or oil and then rocking the razor blade back and forth after the manner of a rocking horse. The first attempt at embedding material appears at this time. Unger tells us that Fenzl devised a crude embedding process for sectioning seeds by dropping the material to be sectioned in a hole made by a hot needle in a piece of stearine, and then the whole mass was cooled and cut. Griffith and Henfry modified this process by substituting white wax as the embedding medium. Schleiden devised another embedding method of immersing minute objects in a thick mucilage of gum arabic which was dried on a small board until glassy. After sectioning the sections were placed in water to swell them to their normal size. Staining was not used in the microtechnique, if we exclude the use of iodine. The article entitled "ovules," an English description of German methods by Griffith and Henfry, gives a good idea of the free-hand methods in use at that time. Their directions

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are as follows, "The ordinary plan is to place an ovule between the thumb and fore-finger of the left hand, and with a very sharp razor cut it into two unequal parts, in the direction of the axis. The larger piece is then laid on its flat side on the finger (by the aid of a mounted needle) and another section made so as to leave a section preserving all of the central part of the ovule. This adheres either to the finger or to the razor and a drop of water should be placed on it to free it; then it may be transferred to the slide by a very fine camel's hair pencil. Examined under a low power it will probably be found to require further dissection, with exceedingly fine needles, under a simple lens, sometimes mere pressure is of service. We have found ovules which have been kept in spirits easier to dissect; when fresh, the cell membranes are excessively delicate."

The compressorium, an instrument much used by the animal histologists of that time, proved of but little service in botany, while maceration methods were used to a considerable extent for the dissociation of tissues. The earliest process, introduced by Moldenhawer, was the decaying of the wood in a manner very similar to Hill's method. Various strong acids and alkalis were also used for this purpose, the recommendations of Schleiden (1842) being typical, but after the discovery of Schulze's maceration methods little else was used. The first fluid Schulze proposed was a mixture of nitric acid and phosphoric acid but the more recent combination of nitric acid and potassium chlorate has found widespread favor among botanists. Chromic acid was also used for this purpose to some extent, having been introduced by Sanio in 1863.

The mounting media developed by the English microscopists, especially glycerine and glycerine jelly, were widely used by the German botanists of the sixties. Mounting media which were originated by botanists in the latter part of the period under discussion include the use of potassium acetate by Sanio the use of potassium hydroxide by Schulze and Hanstein's potassium hydroxide solution. The methods which were used by the German botanists at that time showed, however, no great advance and between 1860 and 1875 the progress which they made in methods is not at all comparable to the progress which the zoologists were making.

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The Development of the Modern Microtechnique

To properly understand the development of botanical microtechnique it is necessary to review the progress that the zoologists had been making in microtechnique. They soon recognized the value of Clarke's process of clearing in turpentine before mounting in balsam and various improvements were made in the procedure. As a result we find Kutschin substituting creosote for the turpentine, and two years later Rindfleisch cleared his preparations in clove oil before mounting in balsam. In the following year, 1866, Steida made an extensive study of the clearing action of about 30 essential oils, and among others recommended bergamot oil.

Although attempts at staining were first made by microscopists on botanical material, or by botanists, the development of the technique of staining is almost wholly due to the zoologists. I have been unable to find anywhere recognition of John Hill as the real pioneer in the history of staining. Another process, which is essentially similar to staining, is that of Reade who charred plant tissues in order to render them more visible when mounted in balsam. Queckett in 1848 recommended charring or staining with either iodine, fustic or logwood extracts to bring out the structure of plant tissues mounted in balsam. In 1843 Goppert and Cohn used carmine for the study of the cell contents of Nitella. A few years later Hartig (1854), used carmine for staining the cell contents and noted that the nucleus was not stained until after the death of the cell. Osborn also used carmine for his studies on the root-tip of the wheat plant. The data on the history of staining have been collected by Gierke and later by Apáthy. Gierke compares the claims of Goppert and Cohn, Hartig, and Osborn and thinks that Hartig should be given the credit for discovering the process of staining tissues with carmine. Apáthy thinks that the real credit for the discovery belongs to Corti, a work which Gierke apparently overlooked. In my opinion, for reasons given above, Hill should be credited with having first used staining methods in connection with microscopical work. Queckett's work also antedates that of Corti or Hartig, although Corti should retain the honor of being the first to apply staining methods to the study of the contents of the cell. Previous to the study of the early literature, Gerlach was generally

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credited with having first used stains. Although this has been shown to be false, Gerlach's use of stains is important for it was through the adoption of his methods that staining came into general use. Apáthy has made a very apt comparison when he says that Corti and Hartig were the Normans (Norsemen?) of staining while Gerlach was the Columbus.

Experimentation also began on the value of other substances for staining tissues. Waldeyer is held by both Gierke and Apáthy as the first to use hæmatoxylin as a stain. This is incorrect, since Queckett (1848) and Wigand had both used logwood stains before Waldeyer. In 1862 Wigand made an extensive study of the behavior of plant tissues towards coloring matters. This work was a study of the phenomena connected with the staining of plant tissues from the standpoint of the commercial dyer rather than from that of the microscopist trying to bring out structural differences. Besides cochineal he stained with hæmatoxylin and certain other colored plant extracts, as yellow wood (old fustic), cutch, and root extracts. Negative results were obtained with indigo and various mineral stains. A selective staining of different parts of the plant was noted but no use was made of this except to help elucidate the theory of staining that he proposed. The first hæmatoxylin stain of real value was that of Boehmer, who, in 1865 gave a formula that is in use to the present day.

Soon after the discovery of aniline dyes, we find the application of them to the staining of microscopical preparations. The first to use them was Benecke, although at the present day we do not know what his "Lila-Anilin-Farbe" represents. In the next year (1863) Waldeyer employed Rosaniline (Aniline Red), Anilien (Aniline Violet), and Parisian Blue (Aniline Blue). In the same year there was the independent discovery of the staining power of Magenta (the English name for Fuchsine) by Lynde and by Roberts. Lynde used Magenta for staining the contents of plant cells and noted that the contents were not stained until after the death of the cell, while Roberts stained blood corpuscles with Magenta. In the following year Abbey experimented with a still larger number of dyes. In studying the contents of the plant cell he used Mauve, Hoffmann's Green (Iodine Green), Aniline Brown, Picrate of Aniline, Magenta, Aniline Green and two blue colors the names

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of which are not given. Except for the work of Lynde and Abbey practically all of the early work on staining with aniline dyes was done by zoologists. The list of discoveries along this line might be prolonged indefinitely; for the first twenty years following the introduction of staining with aniline dyes by Benecke, Gierke cites 55 references, each of which contains something that constitutes a distinct advance over methods known up to that time, and all but three refer to zoological or medical publications. Among the stains most used by botanists today may be noted the introduction of Dahlia by Huguenin in 1874, Eosin by Fischer in 1875, Methyl Violet, Iodine Violet and Safrain by Ehrlich in 1877, Bismarck Brown by Weigert in 1878, and Methylene Blue by Ehrlich in 1881. It must be remembered that these stains were used singly and not in combination as we now use them. The first double staining is that of Schwarz, who in 1867 stained his material in carmine and then in picric acid. Other early combinations of stains were Eosine and Methyl Green, Eosine and Dahlia, and Eosin and Methyl Violet. The method now most generally used, that of overstaining in a solution of the dye and then destaining to the proper intensity became well known through the work of Flemming (1881), although the process had been previously used by Bœttcher and Hermann. Flemming experimented with a large number of dyes to find which one gave the best nuclear stain, among those used were Safrainin, Magdala Red, Dahlia, Mauve, Rouge Fluorescent, Solid Green, Ponceau, Fuchsine, Eosine, and Bismarck Brown. Later Flemming (1884) found another good stain in Gentian Violet. Safranin and Gentian Violet as a double nuclear stain was not suggested by Flemming as is frequently stated, but was first employed by Brazzola; while the much used triple stain of Safranin, Gentian Violet, and Orange G was combined by Flemming in 1891.

The development of embedding methods is also due to zoologists. The term embedding should be used with care since it has been used by investigators in two different senses. In one case there is merely the surrounding of the material with any medium, the Fenzl method mentioned above being an example. In the other embedding process the tissue is completely saturated with

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the embedding medium. The first process might well be called an enclosing method, while Apáthy has suggested the term interstitial embedding for the latter. Paraffine was introduced into microtechnique, as an enclosing medium, by Klebs in 1869. Other substances used for the enclosing method of embedding were gum arabic by Heidenhain, glycerine jelly by Klebs, and albumen by Bresgen and Calberla. In the enclosing methods of Strickler and of Born the material was dehydrated, cleared with an essential oil and then placed in a mixture of wax and oil, while after sectioning some solvent was used to remove the enclosing medium.

Although Schleiden's method introduced in 1842 was an interstitial embedding process the first serviceable method is that of Flemming, who in 1873 used transparent soap. Bergamot oil was used as the solvent for the paraffine in the older embedding methods, but it has since been shown that paraffine does not dissolve in bergamot oil to any appreciable extent. Turpentine as a solvent for the paraffine was not generally adopted since it caused plasmolysis. The independent discovery of cholorform as the solvent for the paraffine by Griesbrecht and Bütschli, in 1881, brought the method up to a point where there could be an interstitial embedding of the most delicate tissues without plasmolysis. The other embedding medium which is widely used today, celloidin, was introduced by Duval in 1879. Duval used collodion, but its use was abandoned after Schiefferdecker showed the greater adaptability of the patent collodion called celloidin.

Fixing solutions are a comparatively recent development. The older investigators were more anxious to obtain some substance that hardened the tissue than to obtain what we now call fixation. The different editions of Lee reflect well this change in attitude towards the hardening agents. Chromic acid was one of the earliest hardening agents introduced, Hannover using it in 1840. The term fixation came into use in the early eighties and practically all of our fixing mixtures were proposed in the decade of 1880-1890. Lang used mercuric bichloride as a fixing medium either alone, or in combination with acetic acid, picric acid, or alum. Osmic acid, although introduced into microtechnique by Schultze in 1864 was not used as a fixing medium until Flesch combined it with chromic

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acid. Flemming experimented with a number of combinations containing osmic acid and decided that a mixture of osmic, acetic, and chromic acids gave the best result. The first formula published is now called the "weak" formula since two years later in 1884, he gave another mixture of the same ingredients in greater concentration, forming what is called the "strong" mixture. These few citations are not given in an attempt to cover the field but more in an endeavor to show the period in which methods of fixation came into general use.

The general opinion seems to be that the microtome is of fairly recent origin. Thomé states that the greater number of microtomes go back to two fundamental types, the Ranvier and the Rivet microtome. Minot thinks that the first microtome which resembles the modern microtome is the instrument of His, the Valentine double-

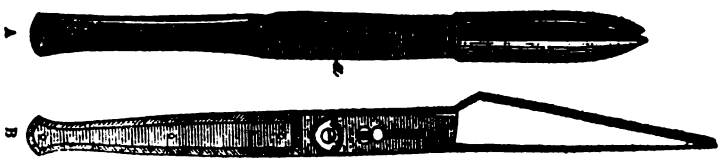

Fig. 14.—Valentine's double-bladed knife. (Queckett, 1848).

bladed knife being regarded as a forerunner of the modern microtome. In discussing the development of microtechnique before 1800 I have mentioned the microtomes of Adams, Custace, and Cummings, while it was also shown that the English microscopists used similar instruments more or less commonly in the years succeeding 1800. Valentine's double-bladed knife (Fig. 14), invented in 1839, has been of limited service in animal histology, and is even sold to the present day. Another instrument, called a micro-

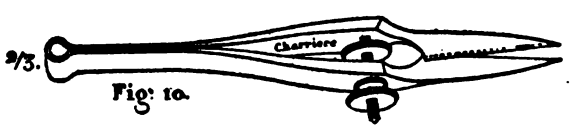

Fig. 15.—The Straus-Druckheim microtome. (Robin, 1871).

tome, is that of Straus-Druckheim. This, as is shown in Figure 15, is really a pair of dissecting scissors, the blades of which are prevented from cutting their full length by means of a screw.

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In 1843 Oschatz invented an instrument for cutting sections which he called a microtome. This term was generally accepted by German histologists but the English microscopists used the term cutting machine or cutting engine, reserving the name microtome for the Straus-Druckheim instrument. Robin has protested against calling sectioning instruments microtomes maintaining that the term should be only employed in connection with the Straus-Druckheim instrument. This is not justified on the grounds of priority since in 1839 Chevalier called the sectioning instruments of Cummings and Adams, "le *couteau micrometrique* ou mieux l' *instrument*

Fig. 16.—Ranvier's microtome. (Dipple, 1882).

microtomique." From this we may judge that the coiner of the word microtome (Chevalier) used it to describe any instrument for cutting sections.

The mechanical principle of Cummings and Adams microtomes is found, with one exception, in all of the microtomes made before 1868. Although varying considerably in detail all of these microtomes have a holder in which the material is clamped and then raised through a cylinder by means of a screw. The Oschatz microtome was made in two forms, either a simple hand microtome or a table form that had a three legged base. Welcker's microtome was very similar to that of Oschatz. Other microtomes which were made in the middle of the last century were those of Smith in 1859, Schmidt in 1859, and Luys in 1868. The microtomes of Nachet and Collin appeared some time before 1870 but I have been unable

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to find the exact date. Other microtomes were also made judging from Harting's statement (in 1864) that there was a very old microtome in the Utrecht Cabinet, while Unger mentions using a cutting machine made by Plossl of Vienna. The microtome of Ranvier (Fig. 16) presents no further advancement in the construction of the instrument, in fact being one of the simplest ever devised, but it was through the use of the instrument by so eminent an investigator that the microtome came into real favor among zoologists. Gudden improved the Ranvier microtome by fastening it to the table, but the fastening of the microtome to the table had been described years before by Topping and by Pritchard and was well known to all the English microscopists of the fifties.

Another mechanical principle used in the microtome is the gradual raising of the object holder by pushing it along an inclined plane. The first application of this principle is generally described to Rivet, but Capanema had made use of the principle twenty years before the invention of Rivet's microtome. Harting is the only one who recognized the value of this instrument and he stated that it was the best microtome which had been made up to that time. Harting reproduces two of the five figures from Capanema's plate but the vertical section is shown upside down so that at a cursory glance there would appear to be little of value in the machine. The instrument was only about 2½ inches long, Fig. 17A, showing a trans-

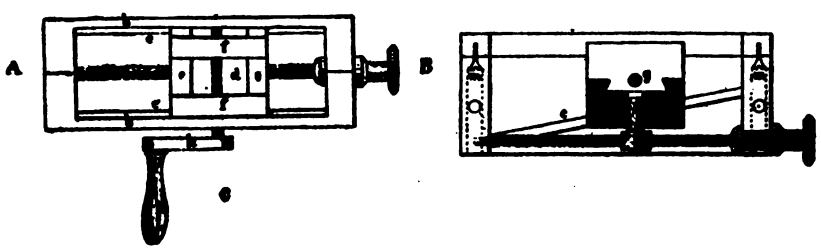

Figure 17.—Capanema's microtome. (1848).

verse cross section, the chief point of interest being the object holder. This consisted of two clamps (ff) which were regulated by a screw (g), a plate (d) forming the base of the holder. After the material had been tightened in place by a crank (k), the crank was removed from the screw. The object holder slid on two lateral

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inclined planes (cc in Fig. 17B) and from its bottom a pillar (1) extended downwards, the turning of another screw (x) moving the object holder. As the screw (x) turned it caused the holder to move up the inclined plane and this slowly raised the material held in the clamp. The sections were cut by means of a knife that slid along two guide plates (bb).

Rivet's microtome (Fig. 18) is the fundamental type on which all improvement of the sliding microtome have been made. Botanists should be interested in this microtome since it was devised for cutting plant tissues. In the advertisements inserted in the 2nd. edition of Nægeli and Schwendener we find Rivet's microtome ad-

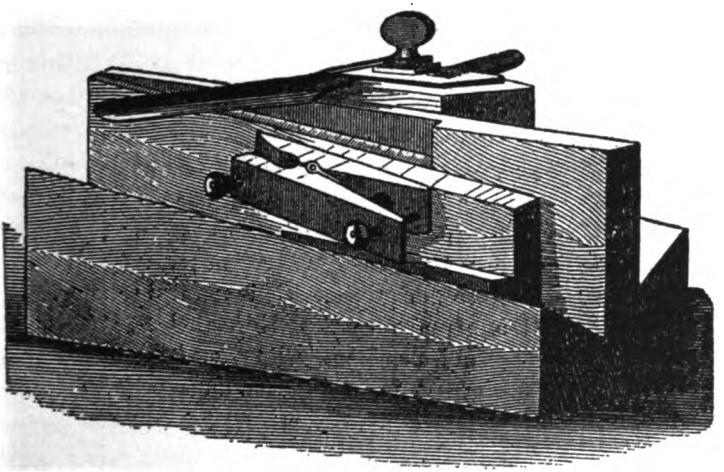

Fig. 18.—Rivet's microtome. (Grönland, 1878).

vertised for cutting plant material, while Ranvier's microtome is listed for making animal sections. The microtome as invented by Rivet was first made entirely of wood by Verick of Paris in 1868. Minot states that it was described in the Annales des Science Naturelles but the first description that I have been able to find is that of Grönland. The microtome consisted of three parts a central block and a separate carrier for the material and the knife. The central block had a base measurement of 16 by 6 cm, and a height of 6 cm. On either side of this block there were wedge shaped grooves so that the middle upper portion was only 13 mm. in thick-

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ness. The groove at the left had a slope of 1:100, whereas the right hand groove was parallel to the top of the instrument. On the top of the middle portion there was a scale parallel to the sloping left hand groove, and this was divided so that each division corresponded to a vertical elevation of 1:100 of a millimeter. A block that was fitted in the right hand groove carried the knife and another in the left hand sloping groove carried the object holder. The apparatus for holding the material was very simple, being fashioned after a patent American clothes-pin. This clothes-pin holder was fastened to the block so that no orientation of the material was possible. The microtome was operated by drawing the knife towards the operator and then shoving ahead the block in the inclined groove the desired distance, the height which the block was raised being computed from the scale at the top of the microtome. After drawing the knife forward the process was repeated.

Brandt constructed the sliding microtome of metal instead of wood, and since this instrument was made by Leyser it is often called the Rivet-Leyser microtome. Grönland considers this substitution of metal for wood a step backwards. A still more important improvement in the microtome is the introduction of the mechanical advancing of the object holder by means of the screw, as found in the Schanz microtome. The other essential improvement was the discarding of the primitive clothes-pin type of object holder and the substitution of a holder capable of being rotated in any direction. Further improvements in this type of microtome have been chiefly variations in already existing principles used in its construction. Perhaps the most notable of these has been the introduction of an automatic device for raising the holder and a mechanical means of operating the knife carriage.

The Caldwell and the Rocking microtome, both types of automatic instruments, were introduced by the Cambridge Scientific Instrument Company in 1885. The Caldwell automatic microtome is of historical interest only, since it was used but little, but in its simplified form, as the Cambridge rocking microtome, it gained a widespread popularity. Since this instrument is so generally known no description of it is necessary. The mechanical error of cutting sections in a curved plate instead of a flat plate has been overcome

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in later models. Ryder's microtome was invented in 1888 but did not come into general use since the mechanical principle underlying the construction of this instrument was also incorrect because the paraffine block did not pass evenly across the edge of the knife but moved in the arc of a very short circle. In spite of this defect one enthusiastic reviewer said that this was "undoubtedly the microtome of the future."

Microtomes of the rotary type are probably the most generally used at the present time. They are an American invention, the idea having been worked out independently by Pfeifer, a mechanican at Johns Hopkins University, and by Minot of Harvard. The first published description is that of the so-called "Johns Hopkins" microtome in 1886. Only one or two instruments of this type were ever manufactured, but it is interesting to note that one of them is still in active service today in the laboratory at John Hopkins University. Minot's microtome was made in 1887 by Baltzer of Leipzig, although the manufacture was soon transferred to Zimmermann, who still makes them. The first published description of these microtomes appeared in 1888.

In discussing the development of the modern botanical microtechnique the problem is largely one of finding out at what particular time methods devised by animal histologists were first applied to botanical problems. To really appreciate how deeply indebted we are to the zoologists it is only necessary to read the names of the fixing solutions and stains used in publications in any botanical periodical. The almost exclusive appearance of the names of Flemming, Heidenhain, Merkel, Hermann, Pianaese, and many other animal histologists in connection with descriptions of methods of study shows this very well.

Moll took 1870 as the date from which he commenced his discussion of the modern botanical microtechnique, whereas Strasburger took 1875 as the starting point for a review of the modern cell theory; but since there was comparatively little development of microtechnique in the decade of 1870-1880, it matters little which date is taken. The article of Strasburger, published in 1875, may be taken as representative of the most progressive methods in use at that time. In the study of cell contents no staining methods were

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used but the necessity of arresting the progress of nuclear division, in order to allow time for a more detained study, was recognized, and absolute alcohol was used as the fixing medium. Permanent preparations were made by transferring the material fixed in alcohol to glycerine. Whenever the material was plasmolyzed by the strong alcohol, a more dilute solution was used for fixation. In some cases iodine was employed as a stain.

That the methods which were being developed at this time by the zoologists were not entirely neglected by the botanists as is shown by the fact that every new method of importance was reviewed in Just's Jahresbericht. Between 1875 and 1880 the most noticable progress in botanical microtechnique was the gradual adoption of staining methods. Among the pioneers may be mentioned Errera who stained nuclei with Nigrosine, Strasburger who used Methyl Green and acetic acid for simultaneous fixation and staining, and the use of Methyl Green by Treub. All of this staining of the cell contents was with a single stain only.

As shown in the discussion of the history of staining most of the very early work with botanical material was to bring out the cell walls. In the same way the first double staining of plant tissues was for the demonstration of fibro-vascular bundles and not for the structure of the cell contents. The development of double staining methods for stems and other tissues was due to the desire of microscopists to make striking microscopical preparations rather than to bring out morphological structures. Some of the earliest work was done by the American microscopists in the late seventies. Among the combinations used, hæmatoxylin and Aniline Blue by Poole, Crawshaw's Aniline Blue and Magenta by Barrett, Carmine and Aniline Green by Peet, while Rothrock used hæmatoxylin or carmine in combination with Iodine Green. Richardson made the most extensive series of experiments on staining among others a triple stain of Atlas Scarlet, Soluble Blue and Iodine Green. The first double staining for the demonstration of cell contents is McFarlane's combination of Diamond Fuchsine and Methyl Green.

During the late seventies other methods used by zoologists were introduced into botanical microtechnique. Leitgeb cleared preparations with clove oil before mounting in 1875; while Parker

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in fixed Chara in a mixture of chromic and osmic acid and after dehydration in alcohol cleared in clove oil and imbedded in cocoa butter.

The best index to the expansion of modern botanical microtechnique is Strasburger's "Botanische Prakticum"; the different editions of which cover a period from 1884 to 1913. At the time that the first edition appeared we find the modern methods of study fairly well begun. The preëminent fixing fluid recommended is absolute alcohol. It is true that chromic acid, concentrated picric acid, and mixtures of chrome-acetic and osmic-chrome-acetic acids are cited in connection with the study of the algæ but since alcohol is the only medium recommended for the study of the anther, ovary, and meristematic tissue the technique of fixation may be regarded as quite primitive at that time. With the exception of MacFarlane's Diamond Fuchsine-Methyl Green mixture, single stains only were used for the study of the cell contents. Carmines and hæmatoxylins appear most frequently in the pages of the first edition although the regressive staining with aniline dyes, so strongly recommended by Flemming in the early eighties, is described in connection with the studies on nuclear division, Safranin and Gentian Violet being considered the best. Double staining is emphasized in connection with the work on vascular bundles and three different processes are described, the use of Grenacher's Alum Carmine-Methyl Green Picro-Nigrosine and Picro-Aniline Blue.

The process of interstitial embedding in paraffine, celloidin, or soap was well known to the zoologists when the first edition appeared and a description of all of these methods is given but no direct application to the study of plant tissues can be found in the book. Strasburger refers to Koch (1874) as having used paraffine embedding in the study of plant tissues, but this method was one of enclosing rather than interstitial embedding.

The chief advance as recorded in the second edition is the use of a much larger number of stains for the study of the plant cell. There is also a much more complete discussion of the process of embedding, but, with the exception of a note on the arranging of serial cellodin sections of anthers there is again nothing said about the application of embedding methods in botanical work.

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To Francotte belongs the credit of introducing the paraffine method into botany. It is true that the notes on the methods for the study of leaves, stems, anthers, ovaries, and fungi cover but three pages but this is the first case where complete schedules are given for the different processes leading to the embedding of plant tissues in paraffine. Francotte did not think that paraffine was the best medium for stems and leaves, but gave the preference to soap, with cellodin as the alternative. Apparently this work has been entirely overlooked, since neither Schönland, Moll, nor Koch mention it. The years 1887-1892 mark the establishment of the paraffine. Attention was called to the usefulness of paraffine through the publication of Schönland and Moll appearing in the year 1887. The first method Schönland used was the gradual dehydration with methyl alcohol and then a transfer to clove oil and from the clove oil to oil of turpentine; afterwards the tissues were placed in paraffine with a melting point of 45 degrees Centigrade. Later (1888) he substituted ethyl for methyl alcohol and also interpolated a further step by allowing the material to remain in turpentine saturated with paraffine before the transfer to pure paraffine. Moll's method differs very little from that which we now use, the chief divergence from the present schedules lies in a much slower dehydration, several hours being allowed for each grade of alcohol into which the material was placed. Campbell and Koch helped to propagate the doctrine of embedding, by showing its applicability to large numbers of plants. With the appearance of Koch's work we may consider the availibility of the paraffine method for botanical microtechnique as well established, although many articles published at that time continued to give full directions for the process.

Historically the soap method is older than that of paraffine, but in their introduction into botanical microtechnique the two are coincident. Pfitzner's description of the soap method in connection with plant tissues appeared the same year as Schönland's and Moll's articles, but the greater adaptability of paraffine has prevented the widespread use of soap although Wilcox and Osterhout are advocates of its use in special cases. The first complete description of cellodin embedding for plant tissues is that of Busse. This substance has always been used to a large extent in section-

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ing woods the most generally used method at the present day being the so-called "Harvard Method" described by Plowman.

The dominance of the school of cytologists led by Strasburger has been most strongly felt in Germany, England, and America. The disciples of Strasburger have generally advocated the mastery of a few methods and after the time of his publication of the process of embedding in paraffine and staining with Flemming's triple stain (1896) this has come to be regarded as the universal method in certain quarters. In other centers, notably the French botanists under the leadership of Mangin and Guignard, there has been the use of a much wider range of stains. At present it may be said that there is a general drift towards a more varied attack. This is perhaps due to the increasing interest in the cytology of the Cryptogams, in which there is a less uniform behavior towards the triple stain of Flemming than there is in the Spermatophytes. This has led to the use of Pianæsæ's stain, Azur Blue, and several others. On the other hand the investigation of cellular organization as exemplified in the study of mitochondria, has called for the development of a special staining technique. With the opening up of these new fields we may confidently look forward to further developments along this same line.

Methods of fixation have not multiplied as rapidly as the methods of staining. A few new fixing fluids have been proposed by botanists, that of Juel being an example; while the French and Belgian botanists have taken several mixtures devised by zoologists and recast the formulæ so that the fluids can be used for the study of plant tissues. On the other hand there is also a strong tendency toward a critical examination of the action of fluids in general use at the present time, the work of Fischer and Lundegard being typical examples.

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THE AMPULLÆ OF LORENZINI IN ACANTHIAS VULGARIS*

By H. E. Metcalf

Introduction

In the past thirty years a considerable amount of work has been done on the lateral line system in the Selachians, in connection with which the ampullæ of Lorenzini have been described. These ampullæ occur in the Selachians only, and for that reason are important, as other Ichthyopsida have lateral line systems which show no traces of these ampullæ. In the Selachians the ampullæ have almost invariably been classed with the lateral line system. There is a great difference of opinion among investigators as to the exact function of these structures; whether they merely do the same work as the lateral line system, or whether they have a special function which is lacking in the rest of the lateral line system. These differences of opinion were not confined to the ampullæ alone, but the lateral line system itself was a basis of dispute. The lateral line system was at first held to be purely secretory, but later was believed to have sensory as well as secretory functions. This opinion is at present generally accepted, but the exact function of the ampullæ is still in doubt, and there are many conflicting statements regarding them.

According to all authors these organs were first described by Stephan Lorenzini in 1678. Since that time many papers have dealt with the subject, most of them treating the ampullæ only superficially, being good for the location of the organs only. Leydig (52), and Boll (68) said that these ampullæ should be classed with the lateral line system. They also saw in them structures which led them to say that the ampullæ were homologous with the electric organs of the torpedo. But the discovery of ampullæ in that fish soon disproved that theory. In 1852 Jacobson had said that the

*The work for the following paper was done at the Biological Laboratory of Tufts College, and at the Harpswell Laboratory, South Harpswell, Maine, under the direction of Dr. J. S. Kingsley and Dr. H. V. Neal, to whom I am indebted for many helpful suggestions during the course of the investigation.

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lateral line system was sensory in function, and that the ampullæ belonged in the same category. Later upon a closer examination of the organs he admitted that the old hypothesis of a secretory function was correct, and that, as the ampullæ were so closely related to that system, they also were secretory in function.

In 1852, both Leydig and Müller published their works on the anatomy of the Selachians, in which they ascribed a secretory function to the ampullæ. This hypothesis was supported by a new work of Leydig in 1868. Considering the technical and optical methods of the time, the results of these two men were truly remarkable, and for a period of nearly thirty years little or nothing was added to the subject; while the belief that the ampullæ were secretory prevailed.

Boll in 1868, and Todaro in 1870 were responsible for the name of Lorenzini'sche Ampulle as applied to these organs.

The histological structure has never been thoroughly worked out, except by Forsell (98) who was not certain of his results, because only material preserved in formalin was available for his use. His paper has an excellent review of all the literature on the subject up to 1897. Peabody (97) published a short article on the innervation of these ampullæ as shown by means of methylin blue preparations. None of the workers have found mucus cells in the epithelium of the ampullæ, and only Parker (10) has done anything in the way of experimental evidence as to their function.

The ampullæ in Acanthias vulgaris are arranged in eight definate groups; two on the dorsal surface (Fig. 1), four on the ventral (Fig. 2), and two on the lateral surfaces. (Fig. 3-A). As might be expected, these groups are bilaterally symmetrical. The position of six of these groups is approximately visible in an external view of the snout, as the openings of the ducts give an indication of the internal arrangement of the organs. Thus on the dorsal side of the snout are a pair of triangular groups of openings. Dissection shows these to be connected with a pair of triangular groups of ampullæ (Fig. 1-A), these groups being smaller in extent than the area occupied by the external openings. From these ampullæ the ducts radiate in all directions, except toward the ventral surface.

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On the ventral surface of the snout, in front of the mouth, the openings of the ampullæ are evenly distributed over the entire ventral surface. (Fig. 2). On dissecting away the skin, however, it is seen that the three cartilege bars of the rostrum, which run almost straight forward, divide the ampullæ into four rectangular groups. (Fig. 2—1, 2, 3, 4.) Here as in the dorsal surface the duct run in all directions, forward, laterally, back and ventrally,

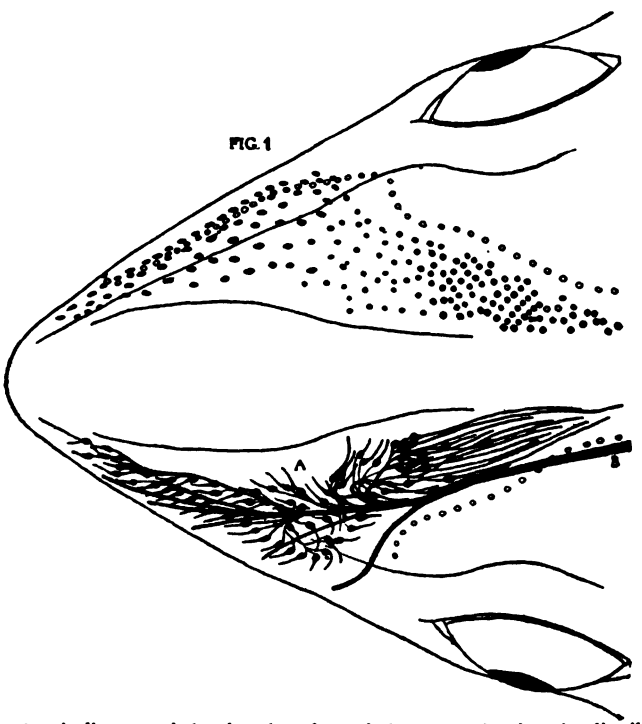

Fig. 1. A diagram of the dorsal surface of the snout showing the distribution of the openings on the right, and the approximate location of the ampullæ on the left. About natural size.

with the posterior ampullæ of the two lateral groups running posteriorly to open at the extreme corners of the mouth. (Fig. 2-B).

On the side of the head, posterior to the mouth, and between the eye and the first gill slit, occur the spiracular ampullæ (Fig. 4-A), all of which have long canals. These open at the posterior

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region of the mouth, and in the line of openings just anterior to the first gill slit.

The two groups on the dorsal surface of the snout are innervated by the opthalmicus superficialis (Fig. 1-B), those of the ventral groups by the buccalis (Fig. 2-C), and those of the spiracular region by the mandibularis externus (Fig. 4-B) branches of the seventh, or facial nerve. These branches also innervate the lateral line system in those regions.

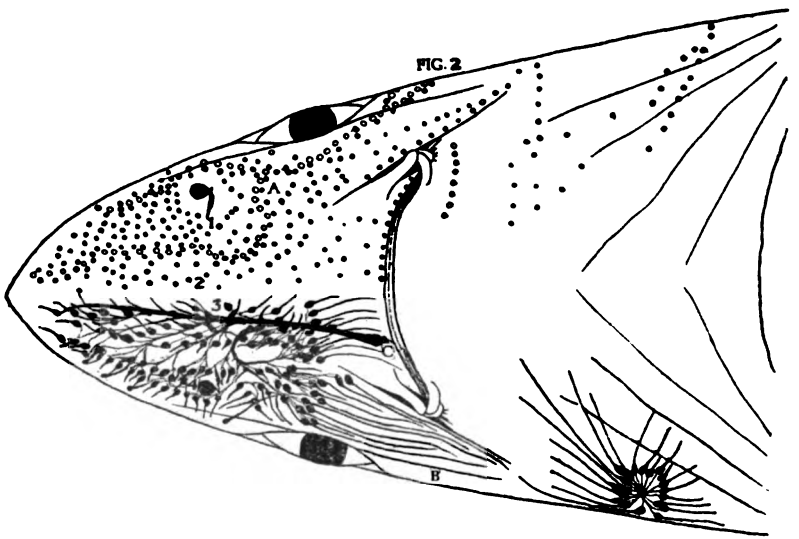

Fig. 2. A diagram of the ventral surface of the snout showing the distribution of the openings on the right, and the approximate location of the ampullæ on the left. About natural size.

The number of ampullæ is not constant, there being from 1,200 to 1,900 in each adult fish. The number in the late stages of the "pup" is approximately the same, so that there is no addition to the number after the fish is born. There are about 500 dorsal ampullæ, 900 ventral and anterior to the mouth, and 200 lateral and ventral, posterior to the mouth. There are no ampullæ posterior to the first gill slit.

All of the ampullæ have definate positions in the head, and in each group the ducts run forward, laterally and backwards as well

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as in all intermediate directions. Some of them also point up, and some down, so that there are ducts radiating in all directions, giv-

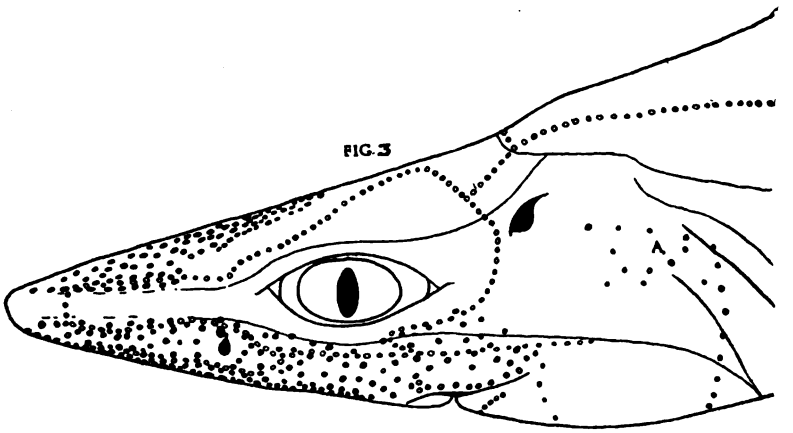

Fig. 3. A diagram of the lateral surface of the snout showing the distribution of the openings. About natural size.

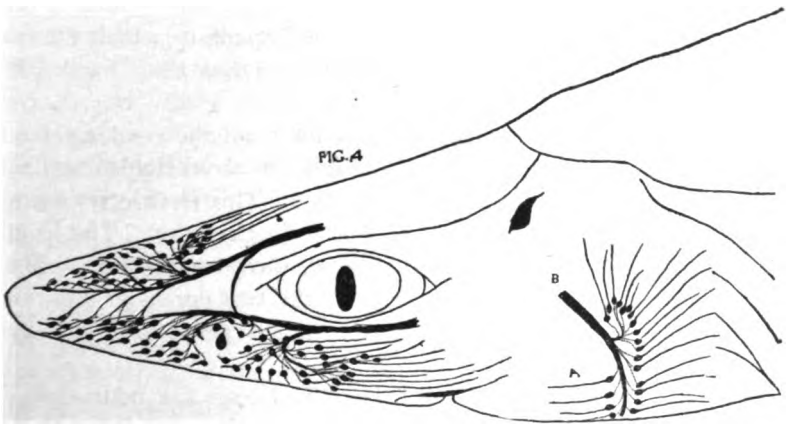

Fig. 4. A diagram of the lateral surface of the snout showing the approximate location of the ampullæ. About natural size.

ing a chance for the reception of a stimulus from any side, above or below. This will be referred to later in discussing the exact function of the ampullæ.

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This problem of their function is an interesting one. They are full of mucus, yet no mucus cells have been clearly demonstrated. Again, typical nerve termination cells which appear to be sensory in character, have not been found. The ampullæ of Lorenzini may be sensory, secretory, both or neither. The main problem then of the present investigation is to find out which of these hypotheses is the correct one. First to see what results might be had by using the experimental method, and then to see if the histological structure of the ampullæ supports those results.

The Gross Anatomy of an Ampulla.

The external openings of the ampullæ are usually single, and nearly circular, although there are cases found where the openings are double, when the two ducts connect with one ampulla. When a double opening occurs the two openings are in the form of semi-circles, so that the size and contour of the double openings is much the same as that of a single one. In the case of a single opening, the main or primary duct extends back to the ampulla itself, but when there are two openings the double ducts are parallel and closely approximate. (Fig. 16-A). Never did I find a case where there was a double duct from one ampulla in which the two tubes ran in different directions to the surface as Forsell (98) mentions.

Each duct leads directly downwards from the opening for a short distance, and then widens out quickly to about twice the diameter of the terminal portion. (Fig. 15-A). This is true no matter whether the main duct is single or double in character. The length of the enlarged ducts varies from six to thirty millimeters. They are longest in the posterior ampullæ of the two dorsal groups, and in the posterior ampullæ of the two lateral groups, ventral and anterior to the mouth.

As the duct approaches the ampullary end the main duct is constricted to about three-fourths its former diameter (Fig. 15-B), and the duct divides into two secondary tubules. These immediately subdivide into two or more ducts of tertiary order on the ends of which are the pockets or alveoli (Fig. 15C), these varying in number on the various tertiary tubes. Some of the alveoli, however, lead directly into the main duct. A careful study of

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those cases in which there are double ducts leading to the surface, as described above, shows that these are to be regarded as greatly elongated secondary ducts (Fig. 16-A), as there does not seem to be any evidence of a branching which would be called tertiary if these ducts were regarded as primary. The alveoli are not on the same level, so that a section through any one plane would not show all of them. This explains why so many of the writers have stated their numbers to be less than is actually the case. There are partitions between the alveoli, so that the lumen of the ampulla is divided into as many compartments as there are alveoli. In about a dozen ampullæ the average number of alveoli was twenty-two, the range of those counted being between eighteen and thirty-two, the majority being from eighteen to twenty-six. The alveoli are elongate in the direction of their major axis, but circular in outline, with their greatest width near the middle. They differ essentially from those described by Boll (68) and Peabody (97) in that Acanthias has no "centrum" cap to which the partitions run. Here the partitions merely separate the alveoli from one another, terminating at the distal end as sharp ridges. The average diameter of the ampullæ in Acanthias is greater than that of six tenths of a millimeter recorded by Peabody for Galeus, as the approximate diameter of those in an adult female about four feet long was; opening one millimeter, duct two millimeters, and ampulla one and six tenths millimeters. The number of ampullary alveoli is also greater in Acanthias than in Galeus or Mustelus.

Do the Ampullæ of Lorenzini Respond to Outside Stimuli?

The experiments carried on by Parker (1910) in his paper on the influence of the eyes, ears, and other allied sense organs on the movements of the dogfish *Mustelus canis*, were repeated in so far as they related to the ampullæ of Lorenzini in *Acanthias vulgaris*. The experiments were carried on in a floating car six feet square and four feet deep. The sides and bottom of the car were covered with wire netting. The fish were mostly adults which had been caught with a hook, and carefully brought to the car in a barrel of sea water. All of the fish in the car which were experimented on were in good condition, and seemed to be fully normal. They

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soon got used to their cramped quarters, and seemed in every way to be fit subjects for experimentation.

Several of the fish were experimented on with more or less success. The first thing tried was to cut the nerves leading to the ampullæ, and to observe the fish swimming around in the car to see whether or not their behavior differed from the other uninjured occupants of the car in any way. Of a number of fish treated in this way, including a number of young "garters" as well as adults, not one of them, as far as could be observed, suffered any inconvenience from the loss of the use of these organs, or any deviation from the behavior of other normal and unhurt fish in the car. In fact, the fish which had had the nerves that lead to the ampullæ cut, could not be distinguished from those that were normal, as they swam about in the car.

Following this, a variation of the same experiment was tried. The fishermen in the vicinity of Casco Bay, Maine, have been bothered so greatly in their fishing by the dogfish, that the custom has prevailed of mutilating the fish when caught so there will be no chance of their ever biting a hook again. These fisherman vouch for the fact that if the tip of the nose be cut off, and the fish be put back into the water, it is unable to sink and flounders around on the surface. A number of experiments were performed to see if there was any basis for this statement, and if it had any relation to the loss of the ampullæ which were in that region, but with negative results. The noses were cut so that all or part of the ampullæ were removed, and in some cases even the brain was exposed, but the fish seemed to be able to sink at will, and behaved in this respect as a normal individual. There seems to be no truth in the statement that a dogfish is unable to sink beneath the surface when the ampullæ are removed, or even when the entire tip of the nose is cut away. The explanation of the fishermen's statement lies in the fact that a dogfish when brought into the air immediately begins to swallow air in gulps so that the intestines are filled. Then when thrown into the water the air inside keeps them at the surface whether or not the tip of the nose is cut away. All operations on the fish were carried on under water so that there was no chance for them to take in air. The ampullæ of a single side, both dorsal

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and ventral, were removed, but the actions of these fish could not be observed to differ appreciably from the controls. The openings of the ducts were also covered with paint so that stimulus from the outside might be prevented, but without result.

Finally it was decided that the response, if any, needed more delicate methods to make itself evident. Following Parker, a patch of skin about two centimeters in diameter was dissected away immediately over a bunch of ampullæ, thus removing any possibility of a response to a stimulus from the sensory nerve endings in the skin. The fish treated was an adult female, the most active and well, as also the largest. After a half hours rest given in order that the fish might be fully recovered from the shock of the operation, various stimuli were applied to the ends of the cut canals which had been exposed. A thin delicate needle point was applied to the canal coming from one ampulla, but no reaction could be observed. Possibly there was a reaction, but it was so small that it could not be detected. Then the blunt end of a dissecting needle handle was very carefully applied to a fresh spot, so that there was no danger of touching the wounded edges of the cut skin, at the same time trying to touch the fish with the hands as little as possible, when it was on the surface of the water at the top of the car. This proved to be a difficult undertaking, as the fish was very active, and showed a distinct preference for the bottom of the car. In order that the results could be relied upon, it was thought best not to try to hold the fish at the top of the water, but after a little practice the fish could be made to stand still long enough for its reactions to be seen. Later it was noticed that the reactions seemed to be the same whether or not the fish was held lightly in the hands while the stimulus was applied.

The normal respiration of the fish was from 42 to 48 spiracular closures per minute. A number of stimulations were made, using the blunt point and watching the spiracular valve for an indication of a response. So far as could be noticed this valve did not change its rate of movement, nor was the rate of respiration retarded as Parker states. Upon turning the fish slightly so that the mouth could be observed, a very distinct reaction was noticed. During the normal respirations the mouth is opened, and some of

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the water is taken in there, as well as through the spiracle. The distance the mouth is opened in the fish under observation was normally about one-half inch at each inspiration. The instant a light pressure was applied to the ends of the cut canals with the blunt point, the mouth immediately closed, but the respiration continued without a halt, all of the water being taken in through the spiracle. The pressure, if applied less than ten seconds caused the mouth to be closed all during the time that the stimulus was applied. If, however, the pressure was continued for longer than ten seconds, the mouth would open after 7 to 8 complete inspirations through the spiracle, and the water would again be taken in through both places, although the mouth never opened to its normal extent while the stimulus was being applied. If the pressure then be slightly increased the mouth would close entirely again for about ten seconds, until it seemed that the fish "got used" to the stimulus. In every case when pressure was relieved the fish gave a distinct gulp with its mouth, giving the observer an impression that it was greatly relieved at being rid of the stimulus. All of these responses to pressure entirely disappeared upon cutting the nerves supplying the ampullæ. All of the foregoing shows beyond a doubt that these organs were sensitive to the stimulus of pressure caused by the application of the blunt point to the cut canals of the ampullæ. These experiments confirm Parker in that the ampullæ of Lorenzini respond to pressure, but in no case did I get an inhibition of the respiratory movements which could be noticed, as did Parker in his experiments. The response was easily seen, and occurred in other dogfish in the same manner upon later experimentation. These results would indicate that whether or not there be a secretory function, these organs most certainly have a sensory function.

The Histology of the Duct and Ampulla.

The walls of the duct are lined with a single thin layer of squamous epithelium, with a thin layer of fibrous connective tissue on the outside. (Fig. 5-C). With iron alum hæmatoxylin the nucleus and cytoplasm of these cells stain very darkly, even when extracted so that the nuclei of the other epithelium in the ampullæ are clear. This makes it somewhat hard at first to see the nuclei in the wall of the duct, but careful extraction for that point alone will

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give good results. The cytoplasm of the cell stains very deeply and seems to be somewhat striated in character. (Fig. 6). Near the ampullary end of the duct there are protoplasmic projections extending out from those squamous cells into the lumen. (Figs. 5-G, and 6-B). These were at first thought to be artifacts, and every possible precaution was taken to prevent any artificial distortion of the cells, but the processes are real and can be seen in all well fixed preparations such as those fixed in Flemming's fluid. These processes arise just above the nucleus, and project a short distance into the lumen. The protoplasm immediately below them is vacuolated, and I am sure that these processes are not cilia. (Fig. 6-C). The number of processes on each cell varies and, as a rule, the farther away from the ampullary end, the fewer the number of processes, until finally there is only a convex contour to the cell, and then a normal squamous epithelial cell with no traces of any projections. (Fig. 5-H). Material which had been fixed in Flemming's fluid was used for studying these processes. It was stained in iron alum hæmatoxylin which was extracted except for the chromatin in the nucleus and then a sharp counterstain with acid fuchsin, much more than would be needed for an ordinary counterstain, for these processes are not visible with the ordinary amount of counterstaining. This overstain brings out clearly the vacuolations in the processes, although the rest of the cell is overstained. All kinds of methods were used and numerous stains tried to be sure of these structures, and all showed them to a more or less degree, even those which gave poor preservation. So I think that it is fairly certain that these are real structures and not artifacts. I could get no hint of their function.

These processes continue into the secondary and tertiary tubes, and are on the partitions between the alveoli, but are not found on the epithelium of the alveoli themselves. (Figs. 5-F, 7 and 8).

At the junction of the ducts and the alveoli, the epithelium and the connective tissue continues, the latter showing little change except that it is thicker, a condition continuing over the outer surface of the alveoli. (Fig. 5-B). The epithelium of the alveoli is best seen in longitudinal sections. (Fig. 5). It is thicker than that

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of the ducts and is composed of two types of cells. At first sight there are apparently two layers, as described by Peabody (97), but this is, I think, based on misinterpretation of sections, at least as far as the evidence in Acanthias shows. (Fig. 5-D). The two types of cells differ markedly in shape of cells and position of nuclei. These are (1) larger cells with their approximately circular nuclei near the basal membrane of the epithelium, (Fig. 9, A, E, C, and F.) and (2) smaller cells (interstitial cells) with pyriform nuclei which lie near the free surface of the epithelium. (Fig. 9, G, D, and B). It is this stratification of nuclei which conveys the idea of a stratification of the epithelium, but careful observation, especially in rather thick sections has convinced me that both types of cells extend from the basal membrane to the free surface. (Fig. 9, A and B). The cells of the larger type are so large that there are not many in each section which are cut directly through the center so that both basal and distal surfaces are shown, but these are seen in a number of cases in thin sections (Fig. 9-A), and I think that nearly every cell in the epithelium extends from the basal membrane to the free surface of the epithelium.

The protoplasm of the larger cells is vacuolated (Fig. 9-E), and the chromatin in the nucleus forms a rather coarse network (Fig. 9-C), while the protoplasm in the interstitial cells is rather striated in character (Fig. 9-G), and the chromatin in the nucleus forms a finer network with scattered granules, giving these a much darker appearance. (Fig. 9-D). In no case have I found a cuticle lining the free surface of the alveoli next to the lumen, as Forsell (98) has described for Acanthias.

Innervation.

The different groups of organs are supplied by several branches of the lateralis componant of the facial nerve, as indicated on page 134, a single twig containing from five to ten medullated fibres running from the main ramus to each ampulla. These twigs run to the centre of the ampulla into the space between the alveoli. There they lose their sheaths, and each breaks up into fibrillæ. These pass out between the alveoli, and form a network upon their outer and anterior surfaces. (Figs. 10 and 11).

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Many methods of impregnation were tried in the attempt to discover the method of termination but all were failures, with the exception of intra vitam staining with methylen blue. This, at best, is uncertain, attacking as it does only certain of the fibres and fibrillæ, and one can never be sure that every portion has taken the stain. Several fairly good impregnations were obtained, and these indicate that the fibrillæ had enlargements in their course (Figs. 10, 11 and 14), and that they sometimes terminated with similar end organs. (Figs. 12 and 13). In every case where exact determination was possible, it was found that these enlargements were upon the bases of the pear-shaped cells of the alveoli and in no case were they found connected with the other cells of the epithelium, nor did the nerves extend on to the walls of the ducts.

These facts seem to favor a sensory function for the ampullæ. There are a number of specialized cells which are in a position to receive stimulation, as the pear shaped cells are in direct connection with the mucus in the ampullæ, and have a connection with branches of the nerve supplying the lateral line organs which are certainly sensory in function. The fact that there are no hair cells does not preclude a sensory function as there are numerous instances in which distinctly sensory nerves are not connected with hair cells. The general agreement is that these ampullæ, and the ear as well, are genetically connected with the lateralis system of organs. Hence the fact that Morrill (97) found sensory cells in the dogfish ear which had no hairs, is of especial interest in this connection. Then again there is no need of continuity between nerve and cell, as Morrill also found in his description of the sensory cells in the ear of Mustelus.

The Functions of the Mucus.

In many of the accounts of these organs the figures show a peculiar striation of the mucus contained in the ampullæ and the ducts leading from them, and some authors have discussed the structure and meaning of this appearance at some length. (Forsell 98). In material taken fresh from the dogfish there is nothing of the sort to be seen, but it always appears in the fixed material, but the character of the striation or fibration varies with the reagent

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used. (Figs. 5 and 6-A). These facts lead me to the conclusion that the appearance is really an artifact.

The mucus of the ampullæ is not needed to lubricate the body, as there are numerous goblet cells all over the body for that purpose. Also in the adult fish there is no evidence of a discharge of mucus when alive. The mucus appears when the ampullæ are squeezed, but otherwise it is not seen. That is to say, no more mucus is found on the surface of the body in the region of the ampullæ than at any other point. Apparently there is little loss of mucus from the ampullæ, all that is lost being worn away by friction of the water at the opening of the duct. Therefore the ampullæ need but little mucus to keep the tubes full. If this view be correct it would explain why so little evidence of mucus secretion is found. Treatment with mucicarmin shows very few cells taking the mucus stain. These cells are isolated and are in the same position as the pear-shaped cells, but are somewhat narrower, have smaller nuclei, and have a greater portion of the cell bordering on the lumen. They greatly resemble the pear-shaped cells and in sections stained in the usual way it is impossible to distinguish between the two. I did not succeed in determining whether or not they are innervated. Some of the sections of the ampullæ do not show any of these cells, so it is evident that they are not common. Again it may be said that the blood supply of the ampullæ is very small. This would imply that the secretory function is very limited—merely enough to replace the slight loss, as a large blood supply would be needed for an extended production of mucus.

The ampullæ are situated beneath the skin in the connective tissue, and are connected with the surface of the body by long ducts. It is therefore certain, that if any stimulus is to reach the sensory cells in the pockets, it must either be transmitted through the overlying integument or it travels to them through the mucus. This, then may be the function of the mucus in the ampullæ—to act as a conductor, by means of which stimulation from the outside is to reach the sensory portions of the ampullæ.

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Summary and Conclusions

It therefore appears from both experimental results and from histological structure:

A. That the ampullæ of Lorenzini in Acanthias vulgaris are primarily sense organs.

B. That they have a secretory function in so far as it is necessary to keep up the supply of mucus in the ampullæ and their ducts. This need is small, as there is little loss.

C. That they respond to the stimulus of pressure, and from the fact that the ducts radiate in all possible direction—

D. That the pressure may be caused by currents of water impinging on some of the ampullæ harder than others, thus giving the fish a sense of the direction of the source of the stimulus.

E. That also by means of these ampullæ the dogfish may determine by means of pressure, the depth of water in which it is swimming.

F. That it is also possible that these organs will respond to the deeper notes as these would cause a vibratory change of pressure on the ampullæ.

G. That these ampullæ from their innervation and their function are rightly classed as sense organs along with the lateral line system in the Selachians.

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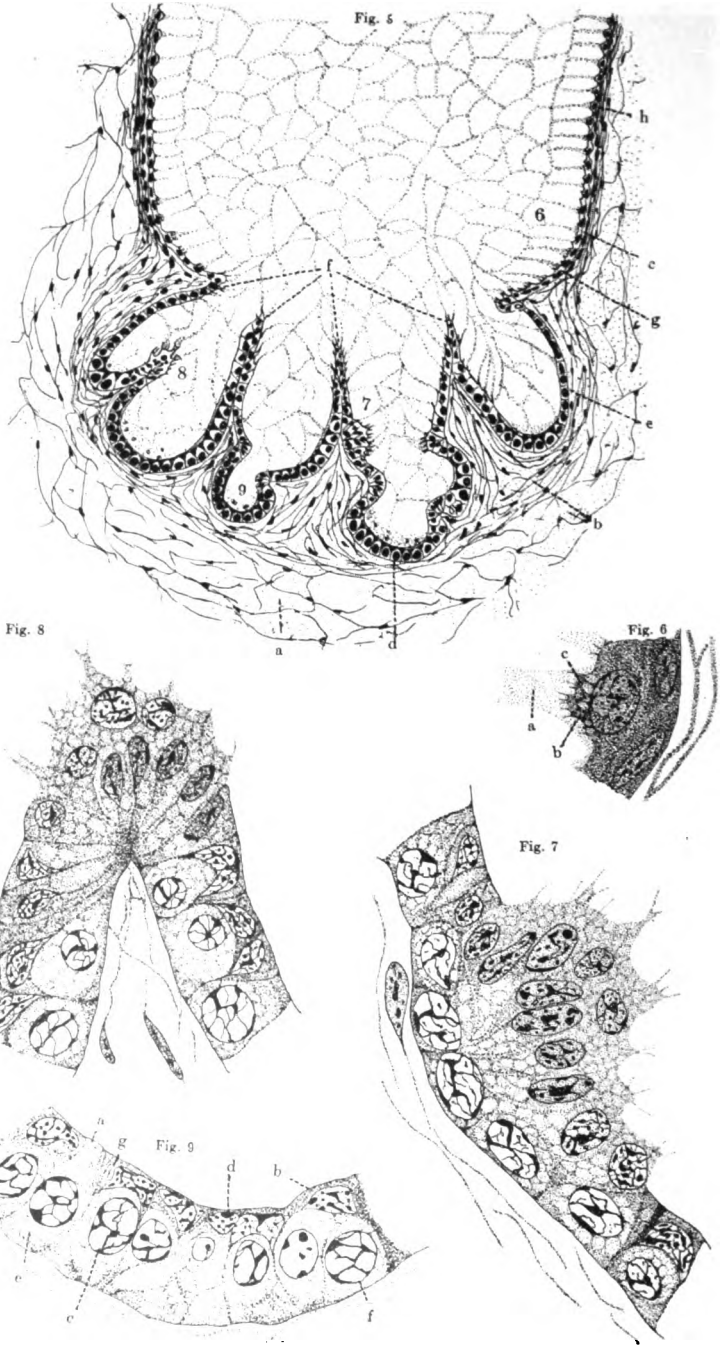

PLATE IV

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EXPLANATION OF PLATES

PLATE IV

Fig. 5. A semi-diagrammatic drawing of a longitudinal section of an ampulla. Flemming's fluid, iron alum hæmatoxylin and eosin. X 200.

Fig. 6. An enlarged portion of the duct showing the protoplasmic processes and the mucus striation. Flemming's fluid, iron alum hæmatoxylin and eosin. X 800.

Fig. 7. A cross section of the protoplasmic processes on the partition between two alveoli. Flemming's fluid, iron alum hæmatoxylin and eosin. X 800.

Fig. 8. A cross section of the protoplasmic processes on the partition between the partition between the alveolus and the duct. Flemming's fluid, iron alum hæmatoxylin and eosin. X 800.

Fig. 9. The epithelium of the alveoli, showing in detail the structure and relationships of the two types of cells present. Flemming's fluid, iron alum hæmatoxylin and eosin. X 800.

The small figures in the lumen of the ampulla shown in Fig. 5 indicate the portions which have been figured in Figs. 6 to 9.

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Plate V

Fig. 10. A thick section through the base of one of the alveoli showing the nerve ramifications over the alveolus. Methylen blue impregnation. X 400.

Fig. 11. A cross section showing the nerve extending laterally after having lost its sheath. Methylen blue impregnation. X 400.

Fig. 12. A thick section showing the method of termination of the nerve fibre on the base of one of the pear shaped cells. Methylen blue. X 950.

Fig. 13. A thick section showing the same kind of termination on a slightly differently shaped cell. This difference in shape and the difference in shape between these cells and the same cells fixed in Flemming's fluid as figured above, may be explained by the fact that the methylen blue process gives poor fixation. X 950.

Fig. 14. A thick section showing two of the pear shaped cells which have a slight enlargement of the nerve fibre at their base, with no termination. X 950.

Figures 6 to 14 are camera drawings.

Fig. 15. A diagram of an ampulla and duct with a single duct.

Fig. 16. A diagram of an ampulla and duct with a double duct.

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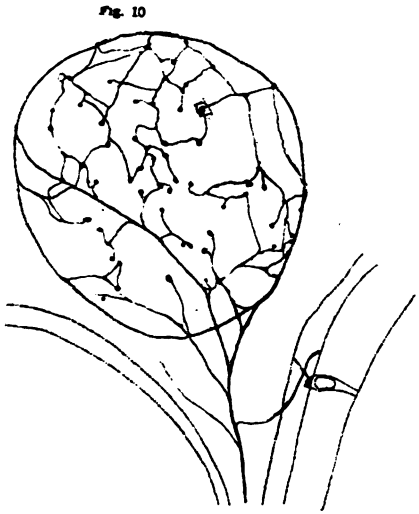

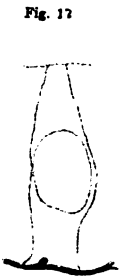

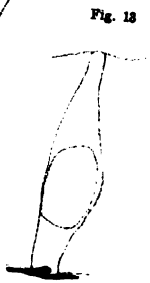

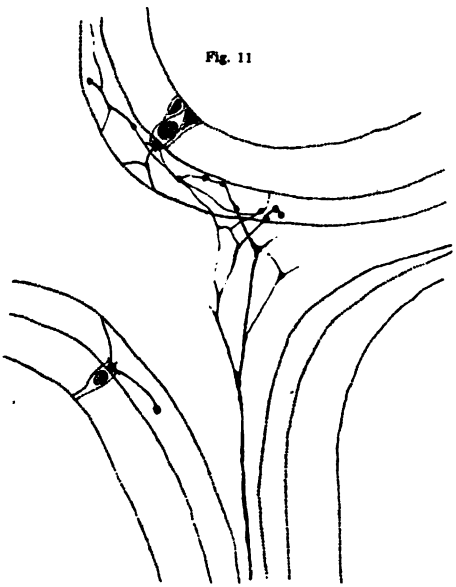

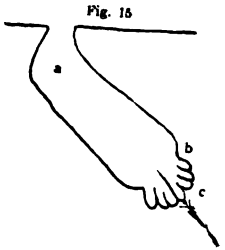

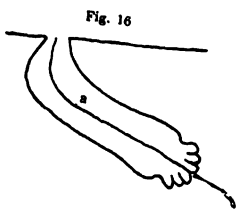

PLATE V

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

PROTOZOOLOGY APPLIED TO THE SOIL

By Nicholas Kopeloff, H. Clay Lint and David A. Coleman

(From the departments of Protozoology and Soil Bacteriology, Rutgers College, New Brunswick, N. J.)

In recent years protozoology has materially widened its scope, as is evidenced by the growing interest in investigations of economic significance such as those concerning protozoa as parasites, in drinking water, etc. It needed but another step to the realization that the protozoa in the soil (and they have been observed in as large numbers as bacteria) may have a definite function in influencing soil fertility. In fact, Russell and Hutchinson[1] contend that protozoa are one of the limiting factors in soil fertility because they feed upon, and consequently limit the numbers of soil bacteria, which are for the most part, beneficial.

While this and other problems are of primary concern to the agriculturist, nevertheless their solution depends largely upon the assistance of the general protozoologist—especially in establishing certain fundamentals in methodology.

Thus at the very outset the investigator is hampered by the lack of an adequate method for counting soil protozoa. The four important methods now in use are:

(1) Rough microscopic examination, which needs no extensive criticism to point out that it is exceedingly inaccurate; (2) Dilution method[2], which is only approximate by virtue of the fact that the

[1]Russell and Hutchinson—Jour. Agr. Sci., *3* (1909): 111; *5* (1913): 152, etc.
[2]Rahn—Centr. f. Bakt. II, *36* (1913): 419.

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differences between dilutions must of necessity be fairly large in rapid work; (3) Loop method[3], where the protozoa in a measured loop are counted microscopically. In the first place the amount of liquid taken up will vary with a number of samples, because of differences in surface tension—especially where various different media are employed. Secondly, the size of the platinum loop itself will vary as a result of heating and cooling (on sterilization in a flame) and chance contact with resistant surfaces; (4) The plating method[4], is necessarily limited, because some protozoa cannot grow on solid media.

It thus becomes evident that the above methods do not lend themselves to accurate work; which fact, led the authors to devise a new method for the counting of protozoa.

This method is an adaptation of the well-known blood-counting apparatus—("Blutkörperzählapparat"—Carl Zeiss-A. H. Thomas, Philadelphia). The principles underlying the use of this instrument is the microscopical observation of a drop of standard size.

The apparatus is designed as follows: A glass disc is cemented to a glass slide in the middle of a circular cavity slightly larger than the disc. A thick cover glass, the planes of which are parallel, is placed over the solution which rests on the glass disc. The excess liquid is pressed into the reservoir surrounding the disc, thus making the film of solution on the disc .1 mm. in depth. It will be seen that the difference in the depth between the glass disc and the cover glass is constant, providing the contact between the solution on the disc and the cover glass is perfect. An area one square mm. in size is marked off on the disc, and this is further subdivided into four hundred squares.

The method of procedure in the use of this apparatus is as follows: Thoroughly clean both slide and cover glass with alcohol. With a sterile platinum loop place a few drops of solution to be examined on the glass disc. It is necessary to have a film of sufficient thickness so that when the cover glass is properly adjusted and slightly pressed down, perfect contact is ensured. The preparation is then placed under the microscope and the organisms are

[3]Müller—Arch. f. Hyg., *75* (1912): 321.
[4]Killer—Centr. f. Bakt. II, *37* (1913): 521.

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examined with either low or high power. With the ordinary Blutkörperzählapparat cover glass it is impossible to use the oil-immersion, but we deem it advisable to have a special cover glass with parallel surfaces ground fine enough to permit the use of that power, because it has been observed that some very small flagellates may escape observation even when viewed with the high power lens.

The protozoa are counted in a number of fields and the average is taken. Examination of each solution was made in triplicate to ensure accuracy. It was observed that the flagellates and ciliates maintain practically the same position in the field for the brief time required for counting. The peculiar movement of each makes them easily recognizable. However, when they are counted in the living state the possibility exists of their moving from one field to another. It depends upon the number of protozoa present, and the importance of the time element, whether or not they are to be counted in the living state.

Because of their rapid motility, the large ciliates are killed before counting. This is done by passing the loopfulls of media through the vapors emanating from a bottle of osmic acid (as recommended by Goodey[5]) before placing them on the glass disc.

If it is desired to stain the organisms, this may be readily done by using equal volumes of solution and stain (e. g. water solutions of methylene blue, gentian violet, etc.) without killing the organisms. Or again the organisms may be killed and stained in one process as with picrosulphuric acid (Kleinenberg); or the same may be done in two separate steps with osmic acid vapor and other stains.

The use of low or high power lenses depends largely upon the kinds and numbers of the organisms present. It is recommended that more fields be counted with the higher than with the lower power.

The results are calculated on the basis of the area under observation to cubic volume, and then per cc. of solution used.

The advantages of using this apparatus for counting protozoa are as follows:

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[5]Goodey—Proc. Roy. Soc. Lond., *84* B (1911): 165.

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(1) It is a direct method thus eliminating many errors attending incubation, etc., and the results can be reported immediately.

(2) It is more accurate than any other method in use, because it is a standard instrument and no mechanical variation is possible.

(3) It is rapid and saves considerable time in contradistinction to other methods, and the technique is simple. For example, triplicate counts on any media were recorded in ten minutes.

(4) The counts check more closely than those of the above methods used.

(5) It can be used to advantage whether the number of protozoa present be large or small.

(6) It can be used for living, killed, or stained organisms, and permits of a thorough observation of the individual organisms.

(7) The experimental error is 5%.

Its disadvantages are that the initial cost is greater than that of other methods, and the sample is too small to be representative. The error of counts is considerable where the protozoa are very few or many in number. A number of fields must be counted because of the uneven distribution, if an accurate count is required.

The starting point for a great deal of experimentation on the activity of soil protozoa is the choice of a suitable medium for the development of the organisms. Little work has been done relative to the comparison of different media, and as a general rule each investigator has a predilection for some particular one which is seldom based upon any study of the advantages and disadvantages of the media adapted to a definite line of research.

Cunningham and Löhnis[6], Killer[4], and others have presented some valuable information upon this subject, and our experiments were modeled to a great extent upon the work of the former.

In order to determine which medium would be best adapted to the large and rapid multiplication of the various kinds of protozoa, the following media were employed in dilutions of .5%, 1%, 3% 5%, and 10%. Dried blood, hay infusion, hay infusion plus .5% egg albumen (Goodey)[5], peptone, horse, cow, and chicken manures (Mar-

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[6]Cunningham and Löhnis—Centr. f. Bakt., II, 39 (1914): 596; ibid. 42 (1914): 8.
[4]Killer—loc. cit.
[5]Goodey—loc. cit.
[6]Löhnis—loc. cit.

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tin)[7], egg albumen, bouillon, and soil extract (Löhnis)[6] in dilutions of 400 cc., 600 cc., 800 cc., 1000 cc., 1200 cc., per kg. of soil.

The results obtained in this survey may be condensed in the following table which indicates the maximum numbers of protozoa appearing on the most favorable medium every day, for five successive days, as determined by the new counting method previously described. It had been previously determined that the count did not increase materially after the fifth day, consequently there was no need of continuing the work for any longer period of time.

Since the object was to determine total numbers of protozoa in the soil, rather than species, no differential count is appended.

Maximum Numbers and Media

Days	Large Ciliates	Large Flagellates	Small Ciliates	Small Flagellates
1	8,520 in Soil Ex. 800 cc.	840 in 10% Hay	4,255 in 5% D. B.	28,750 in 5% D. B.
2	63,800 in Horse 5%	709 in 5% Egg Albumen	9,210 in 3% Chicken	282,000 in .5% Horse
3	319,010 in Hay 10%	10,625 in 10% Hay	208,000 in 3% Chicken	636,500 in Soil Ex. 1000 cc.
4	708,000 in Hay 10%	7,435 in 5% Cow	379,000 in 3% Egg	478,000 in 1% Horse
5	1,410,000 in 10% Hay & Egg	31,900 in 5% Cow	804,000 in 3% Egg	1,878,000 in 3% Hay & Egg

Summarizing the work outlined above, it was found:

(1) The new method for counting protozoa consists of an adaptation of the Blutkörperzählapparat whereby the organisms may be counted directly, rapidly and accurately. It has been used successfully in the comparison of media for the development of protozoa and other experiments.*

(2) 10% hay infusion proved to be the most favorable medium for the development of large numbers of small flagellates, as well as small and large ciliates. Hay infusion in various concentrations with, and without, the addition of egg albumen, proved to be well adapted to the development of the organisms. Hay infusion plus 5% egg albumen proved superior to all other media for the development of ciliates.

(3) Soil Extract is an excellent medium, though somewhat inferior to hay infusion plus 5% egg albumen, and with the soil used in this experi-

[7]Martin and Lewin—Phil. Trans. Roy. Soc. Lond., B. 205: 74.

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ment lower concentrations than those recommended by Löhnis developed protozoa in a shorter period of time.

(4) 3% chicken manure is an excellent medium for the development of small ciliates.

(5) The numbers and species of protozoa which can be obtained from a given soil are largely dependent upon the media employed, time of incubation, as well as the kind of soil used.

(6) In general the order of appearance of protozoa was as follows: small flagellates, small ciliates, large flagellates and finally large ciliates. This is in accordance with Cunningham and Löhnis' observations.

*Note: Further results on experimentation and a complete bibliography on soil protozoa and soil sterilization are awaiting publication.

The Presence of Acidophilous Cells in the Adrenals of Certain Amphibians*

By Thomas Byrd Magath

The *adrenal glands* of the *Amphibians* vary, in color, from a light yellow to a golden reddish-yellow, and extend along the ventral side of the kidneys in a thin band. The function of these ductless glands is not definitely known, altho it is known that an injection of "adrenalin" will considerably increase the blood pressure and that the removal of both glands will result in death, as does an overdose of the extract.

Stilling found in the study of the adrenals of the Rabbit, during the summer, some remarkable cells which stained an intense red with eosin and appeared very granular in structure. He also found cells in *Rana esculenta* which had the same appearance and stained like those in the Rabbit; because of their appearance in the summer he called them *"Summer cells"*.

Patzelt and Kubik, however, found these cells all the year round and because of their affinity for acid stains called them *"acidophilous cells"*. The result of their work is as follows:

(a) There is present in the adrenal glands of *Rana esculenta,* as in the case of Mammals, Birds and Reptiles, two kinds of cells. viz., an epithelial portion and a *chromaffine* portion. The chromaffine portion is made up of large granular cells which turn a brownish-yellow when treated with chromic acid or its salts.

*Contributions from the Biological Laboratory, James Millikin University, No. 12.

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(b) The epithelial portion embraces the common *lipoid-containing* cells, and among these large granular acidophilous cells.

(c) The acidophilous cells appear all the year round and are stainable. They do not change with age, sex activity or nourishment.

(d) These cells are not present in the adrenals of other Anura, Urodeles, or Reptiles.

(e) Acidophilous cells appear in some other glands of internal secretion.

The author, with the purpose of verifying the above results and if possible of extending them, has made a study of the adrenal bodies of five Amphibians, viz., *Rana pipiens, Rana clamata, Spelerpes bilineatus, Acris gryllus,* and *Plethodon glutinosus,* several animals of each species, being used. All of these are different species from those used in the study made by Patzelt and Kubik. It is found that acidophilous cells appear in *Rana pipiens* and *Rana clamata,* as well as in *Rana esculenta,* as reported by Patzelt and Kubik.

The microscopical technique was on the whole the same as used by Patzelt and Kubik, and is given below. The tissue was fixed in potassium bichromate-sublimate-formol, from four to eight hours.

65 cc of 3½% aquous solution potassium bichromate;
35 cc of 5 % aquous solution mercuric chloride;
100 cc of formol.

The tissue was embedded in paraffin and sectioned five μ thick. The best staining was accomplished by hæmatoxylin and eosin. Sections were stained for fifteen minutes in hæmatoxylin and for from one to four hours in a very dilute aquous solution of eosin. The acidophilous cells stained an intense red with eosin, which showed their granular appearance. These cells stained a reddish-violet with the Ehrlich-Biondi triacid stain; indeed they stain with all the acid stains. The chromaffine cells stained a reddish-yellow, due in part to the chromic salt used in fixing. Another stain which was used with good results was iron-hæmatoxylin. The acidophilous cells stained very darkly. The granules stained a deep black while the protoplasm stained a dark grayish-blue. The chromaffine cells stained dark grayish-blue and the lipoid cells, black-gray to a dark gray-blue.

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In general, there are, according to Holmes, two kinds of cells in the amphibian adrenal glands: epithelium cells which are "cortical", are derived from the peritoneum, and chromaffine cells which are "central cells", and regarded by Marshall, as modifications of cells of the sympathetic ganglia. However, in some few species a third kind of cell is found. These are large spherical cells which have a large stainable nucleus and are the "summer cells" of Stilling or the "acidophilous cells" of Patzelt and Kubik.

The results of this study are given as follows:

Rana clamata: Three kinds of cells were distinguished. The acidophilous cells appeared to be greatly in excess of the other cells and were very large, being about 15 μ. The lipoid cells were com-

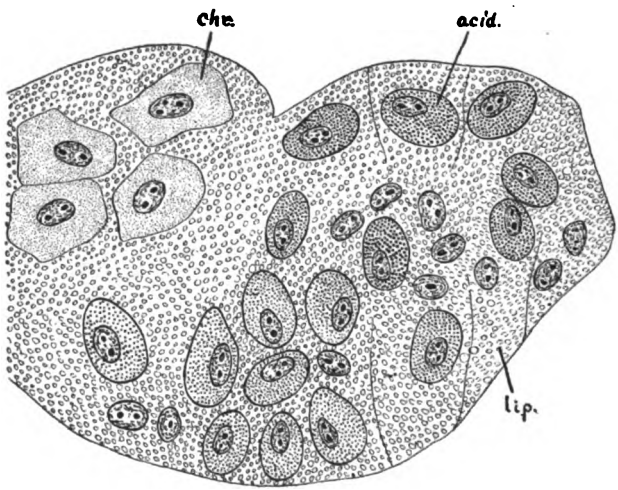

Fig. 1. Adrenal gland of *Rana clamata*

paratively small, with small nuclei, and scattered thruout the tissue. The chromaffine cells were arranged in definite patches and appeared about the size of the acidophilous cells.

Rana pipiens: As in the case of *Rana clamata,* three kinds of cells were distinguished. The acidophilous cells were less numerous and smaller, being about 10 μ. The chromaffine cells were more scattered and the nuclei of the lipoid cells were much larger than in the former case.

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Acris gryllus: Only two kinds of cells were found in this species. The chromaffine cells were quite numerous and large. The lipoid cells were quite a little larger and the nuclei smaller than in the cases of the two frogs given above. No acidophilous cells were found.

Plethodon glutinosus: In this species the lipoid cells are much larger than in any of the other examples, being in some cases 72.5 μ.

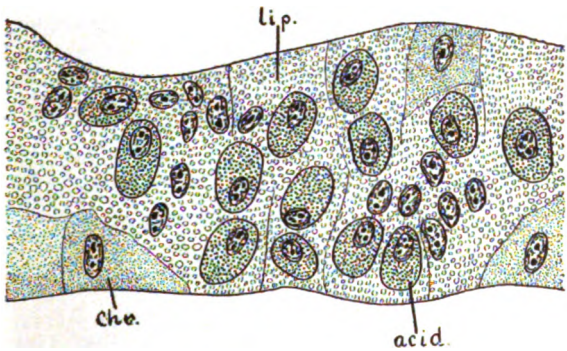

Fig. 2. Adrenal gland of *Rana pipiens*

They are decidedly columnar in shape with large nuclei. The chromaffine cells are not so prominent and the acidophilous cells are lacking.

Spelerpes bilineatus: No acidophilous cells were found in this species. The chromaffine cells were not so large as in the case of the other Amphibians and the cell walls of both these and the lipoid cells stained poorly.

The following conclusions might be made from the observations:

1. As reported by Patzelt and Kubik, in the case of *Rana esculenta,* there appear in the adrenal glands of *Rana pipiens* and *Rana clamata,* two kinds of cells. An epithelial portion composed of the common lipoid-containing cells and among them large granular acidophilous cells, and secondly a chromaffine portion.

2. Acidophilous cells appear in the adrenals of these species all the year round, and should not be called "summer cells" as named by Stilling.

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3. In addition to those Anura and Urodeles named by Patzelt and Kubik, there are no acidophilous cells in the adrenals of *Spelerpes bilineatus, Plethodon glutinosus* and *Acris gryllus.*

4. The presence of acidophilous cells in the adrenals of *R. esculenta, R. pipiens* and *R. clamata* suggests that probably these species are more nearly related to each other than they are to other amphibians which do not have the acidophilous cells in the adrenals. It further suggests that certain physiological activities of these three species are very similar if not exactly alike.

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Millikin University, Decatur, Ill.

June 12, 1914.

TECHNIC FOR CESTODES

LaRue (Ill. Biol. Monog. Vol. 1: Nos. 1 and 2, 1915) gives the following account of methods of treating Cestodes:

The following methods have been used by the writer in the work on this group of cestodes. To a large extent they may be used with success on all groups of cestodes, although it should be understood that certain methods which give admirable results with the relatively small and thin cestodes here dealt with will not give equally good results if used on the large forms such as Tænia.

The larger forms were picked out of the intestinal contents, care being taken to free the head if the worm was attached to the mucosa. These were then repeatedly dipped in the killing solution until the worm ceased to contract. The worm was allowed to lie for 15 minutes to 2 hours in the same fluid. Metallic instruments are to be avoided if corrosive sublimate solutions are used for fix-

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ation. When the smaller worms were encountered the whole intestine slit open was placed in a small quantity of physiological saline solution in a bottle which was then shaken vigorously for about 3 minutes, the killing fluid was added, and the whole then shaken for one half minute. This is according to the method of Looss. The fixative was permitted to act 3 to 10 hours.

The killing fluids used were hot 5% solution of formaldehyde, and hot or cold saturated aqueous solution of corrosive sublimate to which was added glacial acetic acid to make 1 to 2%. Some other fluids were tried but nothing gave better results for the purposes of this study than the corrosive acetic mixture used hot or cold. For most of the worms the cold solution was preferable to the hot which sometimes gave rise to artifacts if used at too high a temperature. In no cases were the worms stupefied before killing.

The usual methods were used for hardening and dehydrating. Specimens were usually preserved in 85% alcohol after running up through the grades. Sometimes after the corrosive acetic fixation 5% formalin was used for a preservative with uniformly excellent results.

Sections were cut 5 to 10 micra thick for the study of histological detail and 20 micra when grosser morphological details were sought. The sections were stained with hæmatoxylin mixtures, either Delafield's hæmatoxylin or Mayer's hæmalum, and decolorized in the manner approved for these stains. Methods of staining in toto followed by sectioning were used with great success at times. For this purpose Ehrlich's acid hæmatoxylin much diluted with 50% alcohol gave the best results. For a contrast stain eosin in 95% alcohol was used on the sections. Acid fushion also in 95% alcohol was sometimes used effectively.

Preparations in toto were much used and were found to be of great value in mapping out the relationships of the organs of the proglottids. Frequently these methods showed everything to be desired except the histology of the organs. In some cases even histological details were well revealed by these methods. The stains which were tried for staining in toto were Mayer's paracarmine, Grenacher's borax carmine, some alcoholic cochineal mixtures, Mayer's hæmalum, Delafield's hæmatoxylin, and Ehrlich's acid hæma-

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toxylin. None of the carmine or cochineal stains were very successful for none of them show the boundaries of cestode structures sharply.

The parenchyma in which the genital organs lie always retained too great an amount of these stains to permit a clear view of the genital organs themselves. The hæmatoxylins, however, usually gave wonderfully clear, sharp pictures of the genital organs. It was at times possible to work out such minute structures as vasa efferentia almost in their entirety from such preparations in toto. The three hæmatoxylin stains were found to be about equally good.

In using these stains it was the practice to dilute the stain with the proper diluent. Relatively large quantities of the diluted stain were used for each lot of material. The stain was permitted to act over night (10 to 15 hours) at room temperature. The excess of the stain was then removed by washing in distilled water and the tissue passed through the grades of alcohol to 70% where it was decolorized rapidly by adding hydrochloric acid to make a 0.5 to 1.0% solution. The object was to remove the stain from the peripheral tissues at a rapid rate and meanwhile leave the stain in the deeper lying tissues. In this method the duration of the acid bath is usually short, depending upon the size of the piece and the character of the stain taken by the tissue, and upon the character of the tissue itself. In general it is desirable to decolorize until a light reddish blue stain remains and until many of the internal structures can be distinguished while the tissue is still in the alcoholic medium. When in the judgment of the operator the proper stain is attained the tissues are placed in neutral alcohol and then into 70% alcohol rendered slightly alkaline by the addition of a few drops of an aqueous solution of sodium carbonate.

Preparations were not flattened but were straightened out on a slide and over this was placed another slide which was supported by strips of paper of such a thickness that little or no pressure was exerted on the specimen by the slides. Dehydration and clearing were accomplished while the preparation was thus kept straight. Xylol and cedarwood oil were used as clearing agents. Preparations were mounted in balsam.

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The methods outlined above yielded very satisfactory preparations for the study of these cestodes and they have also been used by the writer on other cestodes and on trematodes with great success. It is noteworthy that the carmine stains give beautiful preparations of trematodes in toto but fail almost entirely for cestodes. For the cestodes these stains fail because they do not sharply and clearly outline the sexual organs as they do in trematodes, though not better than do the hæmatoxylins. In the judgment of the writer the use of the carmine stains on cestode material has been responsible for many errors in the interpretation of cestode structures.

CULTIVATION OF PLASMODIUM OF BADHAMIA

Hilton (Jour. Queck. Micr. Club, Nov. 1914) describes a method which he has found successful for the continuous cultivation of plasmodia of the Myxomycete, *Badhamia utricularis*. He uses bread which is kept moistened with water. He finds that it stimulates the growth of the plasmodium to use from time to time, instead of pure water, a mixture consisting of a quart of water to which has been added half an ounce each of ammonium phosphate and cane sugar. This seems to give greater vigor to the plasmodium itself, and also aids it indirectly in that it stimulates the growth of the filamentous moulds which grow on the bread and are used by the plasmodium. It would be interesting to know whether this method would serve for other species.

DAPHNIA WITHOUT SEXUAL FORMS

Banta (Proc. Soc. Exp. Biol. and Med., 1914, p. 180) has reared *Daphnia pulex* thru one hundred generations without males and fertilization. There is no apparent decrease of vigor or vitality, and thus the sexual cycle seems not to be inherently necessary, altho males have been found in nature at Cold Spring Harbor.

VERTEBRATE EMBRYOLOGY

In this new work on Embryology, Dr. Prentiss undertakes in one volume to give a working account of the development of the chick and the pig, together with a description of the stages of human embryology, histogenesis, and organogenesis. The figures are

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numerous and beautifully presented; the text is clear and satisfactory. The figures, some of which are in colors, include a large number of whole embryos, dissections, and reconstructions. After a study of these the serial sections can be related to the whole in such a way that the student can readily visualize the plane and level of his section. The book is practically a matrix of human embryology in which are imbedded the special chapters on the embryology of the chick and pig. Numerous statements of a comparative character are included. The treatment is such that the book will be of distinct value and interest to any student of vertebrate structure and development.

The arrangement of the chapters is as follows:—Chapter 1, The Germ Cells; 2, Segmentation and Formation of the Germ Layers; 3, The Study of Chick Embryos, of twenty-five hours, thirty-six hours, and fifty hours; 4, The Fetal Membranes and Early Human Embryos; 5, The Study of Pig Embryos; 6, Methods of Dissecting Pig Embryos,—with further studies upon the development of the head; 7, Entodermal Canal and Its Derivatives (chiefly human, as are the succeeding chapters); 8, Urogenital System; 9, Vascular System; 10, Histogenesis; 11, Morphogenesis of the Central Nervous System; 12, The Peripheral Nervous System.

The writer calls attention to the value of a study of Embryology to the medical student, in that it aids the understanding both of normal anatomy and of the meaning of anomolies. He also insists that the view of medical graduates that it is no longer a fruitful field of research is false. Aborted embryos and those obtained by operation in case of either normal or ectopic pregnancies should always be saved and preserved by immersing them intact in 10 per cent formalin or in Zenker's fluid. An Institute of Embryology has recently been established by Professor F. P. Mall of the Johns Hopkins Medical School for the purpose of collecting, preserving, and studying human embryos. Material may be sent there with assurance that it will be used to the best possible advantage.

An adequate index concludes the book. The mechanical make-up is all that can reasonably be asked.

A Laboratory Manual and Text-Book of Embryology, by C. W. Prentiss, Ph.D. Octavo of 400 pages with 368 illustrations. 1915. W. B. Saunders Co., Philadelphia and London. Cloth, $3.75.

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Secretary.

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TRANSACTIONS
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CELL CHANGES IN THE EPIDERMIS DURING THE EARLY STAGES OF REGENERATION IN THE TAIL OF THE FROG TADPOLE, WITH SPECIAL REFERENCE TO THE NUCLEUS-PLASMA RELATION[1]

By
HERBERT EDMOND METCALF

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I. STATEMENT OF THE PROBLEM

The following paper gives the results of a histological study of the cell changes in the epidermis during the early stages of regeneration in the tail of the frog tadpole, Rana clamitans. It deals with the migration of the epidermis which follows the operation and the accompanying behavior of the epidermal cells, with special reference to the nucleus-plasma relation.

[1]Contribution from the Zoological Laboratory of the University of Illinois, No. 47.

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Fraisse (85), and Barfurth (90) both worked on the reparation of the epidermis and other epithelia in vertebrates, and decided that in the adult amphibian tail the new epidermis arises from the elements of the old epidermis, although no evidences of mitosis were found until the wound surface was completely covered with new epidermis.

Rand (01) found that also in the earthworm there is an epidermal investment of the wound surface, and in a later paper (04) decided that the new epidermis arises by means of a migration of the old epidermal cells, and described to some extent a few of the cell changes which accompany the migration, as does Emmel (10) for the crayfish.

Both of the last mentioned authors speak of a cytoplasmic decrease or an increase in nuclear volume of the cells in the migrating epidermis, either of which would change the nucleus-plasma relation.

The amphibian tail was selected because of the size and character of its cells, in that they are large and easily studied under the microscope. The present paper aims to give an account of the migration of the epidermis to cover the wound, and to describe the cell and nuclear changes in the migrating cells.

II. MATERIAL AND METHODS

Two dozen tadpoles of Rana clamitans averaging 75mm. in length were obtained in September, 1914, kept in an aquarium and fed on saltine crackers while awaiting experimentation. One fifth of the tail was removed and the stump allowed to regenerate for the desired time. When a portion of the regenerated tail had been cut off for study, the tadpole was put back into the aquarium and allowed to regenerate until the process stopped, and then used over again. This method has its advantages, as a section from a regenerated tail is much more easily cut than is the original tail, and makes thinner sections possible because of the softness of the tissues. By a comparison of sections from a regeneration of the original tail, and those from a tail previously regenerated, no difference was found.

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Pieces were taken from tails of 5 minutes, 1 hour, 3 hours, 12 hours, 20 and 24 hours regeneration, fixed in Bouin's fluid, and imbedded in paraffin. A number of fixing fluids were tried, and by far the best results were obtained with Bouin. Sections were made 5μ in thickness, mounted in serial fashion, and stained with a number of different stains. Iron alum hæmatoxylin gave the best results for the determination of mitoses, while Ehrlich's acid hæmatoxylin and eosin furnished the best pictures for general tissue differentiation, as cell boundaries were well brought out.

III. OBSERVATIONS

1. *The behavior of the epidermis as a whole.*

The normal epidermis in the tail region of the frog tadpole is composed of several easily recognizable regions. At the surface I have not been able to make out a cuticle. The outer layer is composed of a definite layer of cells, cuboidal in section, with almost spherical nuclei (Fig. 6A). The cytoplasm at the surface stains slightly darker than that deeper in the cell (Fig. 6E). Next there are from one to five layers of cells with no regular arrangement (Fig. 6B), and a bottom layer of slightly columnar cells (Fig. 6C) with oval or round nuclei which have their long axis, if any, at right angles to the surface of the epidermis. Underneath these cells is a rather thick basal membrane, smooth staining, without fibrilation or striation of any kind (Fig. 6D). This membrane, which is drawn dead black in all of the illustrations, is of great importance in the present study, as it is not regenerated in the early stages, and therefore the place where the epidermis has been cut can be easily seen by determining where this membrane stops.

A number of experiments were tried in order to watch the course of the regeneration in the living material under the microscope, but without material success.

Figures 1-5 show a series of sections near or through the notochord at various times of regeneration, drawn semi-diagrammatically to show the action of the epidermis. Fig. 1 is a frontal section of a tail of a 1 hour regeneration. This shows very clearly the first process which takes place in the regeneration. The muscles have contracted both inward and around the tail, thus decreasing

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to some extent the wound surface. This contraction has the effect of rounding over the square edges of the cut end, and bends the epidermis around on the lateral sides so that the epidermis of the opposite sides touch near the dorsal and ventral sides of the tail where it is very narrow. At these places the epidermis fuses, and thus further decreases the wound area. The epidermis has not at this time begun to move, which is well brought out by the fact that the basal membrane has about the same relation to the epidermis as it had at the time of the cut (Fig. 1B). A clot is being formed to cover the wound, in which black staining fragmenting nuclei of

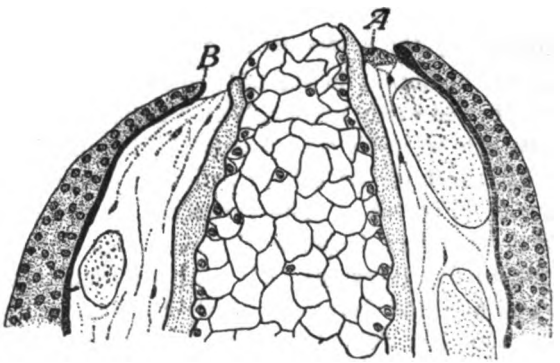

Figure 1

Fig. 1. Frontal section through the notochord 1 hour after the operation, showing the relation of the epidermis to the basal membrane, and the beginning of the formation of the blood clot. A, blood clot; B, basal membrane. (200 diameters)

degenerating cells are beginning to be seen. In most cases the next thing to appear is a complete blood clot over the wound surface, although the time of the formation may be before one hour has elapsed, or later, but in all cases the clot was formed to a greater or less degree before 12 hours had past (A-Figs. 1-5).

Figures 2 and 3 show the period of greatest activity in the epidermis. Figure 2 is a frontal section, and Figure 3 is a sagittal section of a regeneration of 12 hours duration. As will be seen in Figure 2 the epidermis has left the basal membrane behind, and has commenced on its advance over the wound tissues and the clot (Fig. 2B). This plainly shows the importance of having some landmark by means of which one may tell whether or not there has

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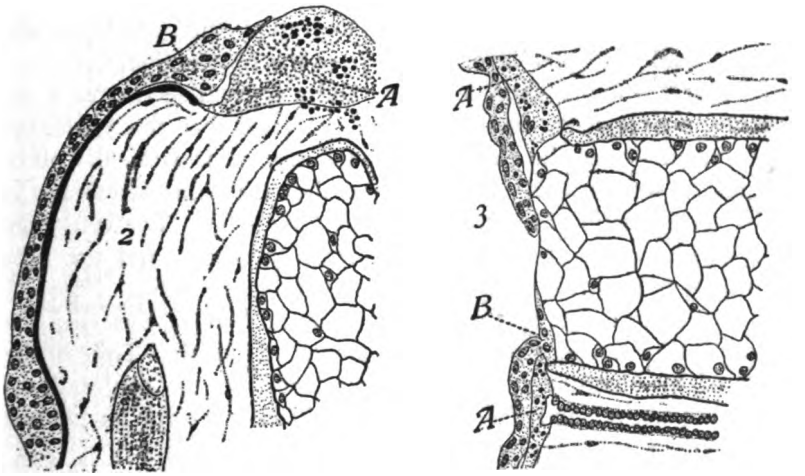
been an actual advance of the epidermis, or whether this apparent advance is only a flap of epidermis left by an incomplete cut which has folded over the wound surface. In the several cases in which the latter happened, it was immediately noticed because of the accompanying basal membrane. In the normal epidermis the cells seem to stick rather closely to this membrane, and in the normal individual when the epidermis is torn off, the basal membrane usually comes away with it.

Figure 2A and 3A show the position of the blood clot over the wound surface. In the tail from which Figure 2 was taken, the clot covers the entire cut surface including the notochord, while in Figure 3, the notochord is not covered. This is a very finely fibrinated clot with a rather thicker fibranation near its outer surface. It is for the most part homogeneous, however, and has in its numerous fragments of the nuclei of the degenerating cells. These fragments arise by a process of amitosis, or fragmentation, as it perhaps had better be called. (Sutherland-15.) At first these



Figures 2 and 3

Fig. 2. Frontal section near the notochord 12 hours after the operation, showing the advancing end of the epidermis, and the blood clot covering the entire wound. A, blood clot; B, advincing end of epidermis. (200 diameters)

Fig. 3. Sagittal section through the notochord 12 hours after the operation, showing the advancing edges of the epidermis and the blood clots on each side of the notochord. A, blood clot; B, advancing end of epidermis. (200 diameters)

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fragments do not stain very dark, and seem to come from the cells near the cut surface, but later they stain almost black, and for some distance back into the tissue immediately behind the cut edge the nuclei take the heavy black stain, showing, in all probability, a degenerative process. One very peculiar thing was found during the early stages, in that while all of the muscle, connective tissue, notochordal, and nerve cord cells for some distance back from the cut show fragmentation and degeneration, not a single nucleus of the epidermis was found to have a degenerative appearance, as evidenced by fragmentation and deep staining.

In the early migration the cells seem to travel in two different ways. In Figure 2, the advancing end of the epidermis is composed of several different layers of cells (Fig. 2B), while the advancing edge of the epidermis in Figure 3 is composed of only one layer of cells (Fig. 3B). It appears to me that all of the cell layers would advance nearly equally as in Figure 2 if the configuration of the surface ahead of it would allow it to do so. The surface in Figure 3 is somewhat obstructed, and as it is the outer layer of cells which is the most active in the migration, the cells below are stopped, and the outer layer passes over the obstruction.

Figures 4 and 5 show a frontal and a sagittal section of a regeneration of twenty hours. Figure 4 shows that the epidermis has advanced over the clot and met the epidermis from the other side and fused with it. It also shows that the basal membrane is in the same position as it was when cut (Fig. 4B). The epidermis here which covers the clot is of normal thickness, and the cells seem to have returned to their normal position and shape. Figure 5 does not seem to be as far along as Figure 4 as in this case the investment of epidermis is only a single cell thick. This is the result of an obstruction of the deeper layers of the epidermis as mentioned above, and the investment will not reform its several layers until the beginning of mitosis at about 48 hours.

One of the first things which is noticed in the movement of the epidermis is the lack of any evidences of cell multiplication in the epidermis, either near the point of movement, or farther back, where mitosis or an increase of cells by any means would aid in pushing the epidermis over the wound. There seem to be even

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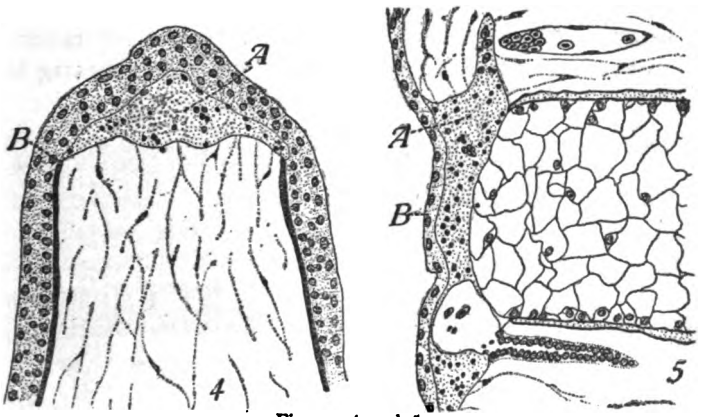
fewer mitoses than normally in the epidermis. In all of the sections which have been examined up to a stage of 20 hours regeneration there was only one mitotic figure found. This was near the region where the epidermis was normal, and had no part in the movement of the tissue.

I will now sum up briefly the movement of the epidermis as a whole. As a result of a strong muscle contraction of the tail during the first hour, the epidermis from the two sides is brought in contact above and below the notochord, thus decreasing the wound



Figures 4 and 5

Fig. 4. Frontal section just above the notochord 20 hours after the operation, showing the wound healed by the meeting of the epidermis from the two sides, enclosing the blood clot. A, blood clot; B, basal membrane, marking position of cut. (200 diameters)

Fig. 5. Sagittal section through the notochord 20 hours after the operation, showing wound healed by meeting of epidermis from the two sides, enclosing the blood clot. Note that here the epidermis is only one cell in thickness, while in Fig. 4 the investment is several cells in thickness. A, blood clot; B, epidermal investment. (200 diameters)

area. The epidermis at these places fuses and plays no further part in the investment of the cut surface. The epidermis surrounding the cut notochord and the thick part of the tail then starts to migrate over the blood clot and wound surface, unhelped by cell multiplication within the tissue. The epidermis from the two sides meets near the center of the cut surface, and the cells return to their normal shape and size. If the epidermis has been able to migrate with all layers of cells, normal stratification is resumed. If only

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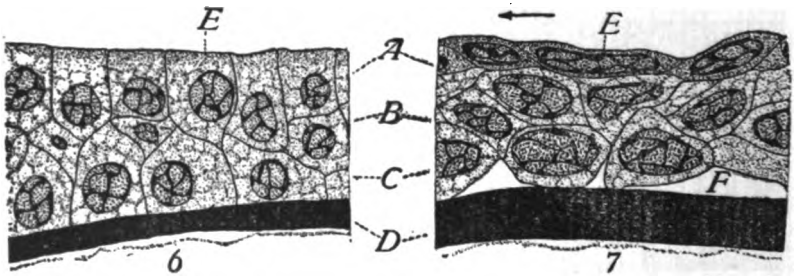
a single layer of cells has migrated over the wound surface the epidermis remains a single cell in thickness until mitosis begins. This action encloses the blood clot which has been formed and is complete at from 20 to 24 hours. The next process which occurs is the absorption of the blood clot and when this is completed, at about 48 hours, regeneration by mitosis begins.

2. The behavior of the individual cells in the epidermis.

As has been stated, the normal epidermis is composed of several definite layers, the most conspicuous of which are the upper layer of cuboidal cells, and the lower layer of columnar cells. There are several layers of cells between these two limiting layers.



Figures 6 and 7

Fig. 6. Section through the normal epidermis showing the relation of the layers of cells. A, outer layer; B, intermediate layer; C, columnar layer; D, basal membrane; E, darker cytoplasm at surface. (1100 diameters)

Fig. 7. Section through the migrating epidermis some distance away from the advancing end, showing the change in shape of cell and nucleus. A, outer layer; B, intermediate layer; C, columnar layer pulling away from basal membrane; D, basal membrane; E, greatly elongated cell at surface; F, space between basal membrane and epidermis. (1500 diameters)

The lower columnar layer is tightly stuck to the basal membrane. In the twelve hour stage of regeneration, which seems to be the stage in which the migratory movement is the greatest, as we leave the normal epidermis and approach the cut edge, the first noticeable thing is that the columnar cells on the basal membrane lean over in the direction of the cut surface, so that their nuclei instead of being at an angle of 90° with the basal membrane, are coming to be more nearly parallel with it (Fig. 7C). At the same time the upper layer of cuboidal cells commences to lose its stratification,

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and the cells change from a cuboidal shape to one which is elongated in the direction of the cut surface (Fig. 7E). In following along the epidermis of a 12 hour preparation, one can find all stages in the change of shape, from the cuboidal to the greatly elongated cell at the place of greatest movement.

Immediately after the basal columnar cells start to lean in the direction of the cut surface, spaces appear between the basal cells and the basal membrane, so that finally the basal cells are in connection with the basal membrane in only a few places (Fig. 7F). These spaces are large and do not appear to be in the cytoplasm of the basal cells, but rather in the region between the cell and the membrane. The nuclei of the basal cells do not appear to change

Figure 8

Fig. 8. Section through the notochord, showing the advancing end of the epidermis composed of a single layer of cells. (900 diameters)

their shape to any great degree, merely rotating through 90° so that their major axis is parallel to the direction of movement. This is not so, however, with the cells of the upper layer. Here the nuclei in the normal epidermis were nearly spherical, so that when the cell elongates in the direction of movement, the nuclei are squeezed into an elongated shape.

After the cells have passed the place where the cut was made, most of their nuclei except those of the upper layer have resumed

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their normal spherical or slightly elongated shape. Those of the upper layer are still in an extremely elongated condition, as are the cells. In some of the cases found, it was only this upper layer which was migrating over the clot surface (Fig. 8). This would seem to show that the upper layer of cells was the one which was doing the most active migration, and that they were pulling along the lower cells. As was mentioned before it is this outer layer which passes around obstructing portions of the wound surface.

In fact, it is only in this external layer of cells that any change in nuclear shape and change in chromatin arrangement can be detected. The nucleus changes from a spherical to a greatly elongated shape. This is due, I am convinced, not to any action of the nucleus itself, but is merely a passive adjustment to conditions laid upon it by the moving cell. However, there does seem to be a change of some kind, for the nucleus takes on a rather deeper stain than it did in the normal epidermis, and the chromatin does not appear to be arranged in exactly the same manner as normally. In the normal nucleus of the outermost cells, the chromatin is in a rather coarse reticulum, differing in no way from that of any of the other cells in the epidermis. After migration starts the chromatin appears to be in a much coarser network than formally, with thick strands of chromatin parallel to the direction of movement. I do not find any evidences of "polarization" as does Emmel (10) in the regeneration of the epidermis in the crayfish. The appearance of these cells under the microscope is one of movement, as they extend their cytoplasm forward toward the cut surface in a pseudopodial manner. In no case did I find any cells positively identifiable with epidermal cells wandering loose. Several isolated cells were found, but were decided to be leucocytes.

After the clot has been covered, whether it be by one or more layers of cells, these outside cells regain their normal shape, and the nuclei come back to the spherical form. Their migratory movement is finished with the fusion of the epidermis of the two sides of the tail. After this fusion and the resumption of the normal shape, there is a resting period of about 24 hours while the clot is being absorbed. During this latter period there is no mitosis, and the epidermal cells have every appearance of normal epidermal

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cells, altho as has been said there may be only one layer. In this connection it may be said that a count was made to determine whether or not there is amitosis in the regenerating epidermis after mitosis has begun, and the resulting ratio was about 100 characteristic mitotic figures to one dumbbell shaped nucleus, which may or may not be an evidence of amitosis. In fact, after a careful study of the later stages of the regeneration, I am inclined to think that amitosis plays no part in the process. Of course, it is hard

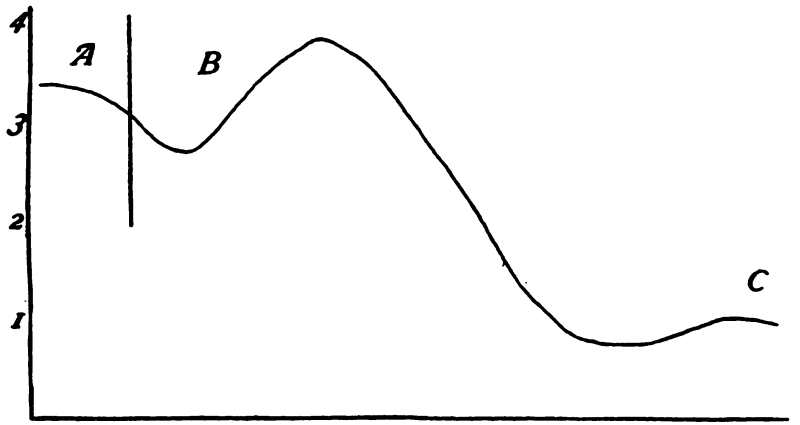

Figure 9

Fig. 9. Curve showing the decrease in the nucleus-plasma relation in the migrating cells, taken from a frontal section. The abscissa represents the position of the cell in the epidermis, and the corresponding ordinate the nucleus-plasma ratio for the cell. A, region of normal epidermis; B, region of the beginning of migration; C, region of the advancing end and active migration.

to recognize true amitotic figures, but from all the observations made there was not the slightest evidence that amitosis was characteristic or even common in the regenerating epidermis.

3. The nucleus-plasma relation in the migrating epidermis.

The nucleus-cytoplasm ratio was obtained by a method described by Dolly (13) in which the measurements for the axes of the cells and nuclei are made from camera lucida drawings at a magnification of about 1000, with a rule graduated to 1mm. The relative volumes are calculated by supposing that the unknown third dimension is equal to the shortest axis as found in a single

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section. These three dimensions are then multiplied together, subtracting the nuclear volume from that of the cell. The resulting figure for the plasma mass when divided by the nuclear volume gives a figure corresponding to Richard Hertwig's conception of the nucleus-plasma ration.

A glance at Figure 9 will show at once some of the results. The curve was obtained by plotting the cell measurements in the relative positions the cells held in the migrating epidermis between the normal portion and the advancing end.

As will be seen from the curve, the nucleus-plasma ratio of the cells in the normal epidermis is about 3.5. The ratio seems to increase slightly as the cells begin to leave their regular positions in the epidermis, and then to steadily decrease until the ratio in the actively migrating cells is about 1.0. These ratios were obtained from a frontal section.

This reduction is extremely interesting if the results of such a measurement can be relied upon. Of course it is not necessary to have an accurate measurement of volume, it being only important that the calculations apply to all of the cells without a variation due to the changes in the shape of the cells. It may be interesting to note in this connection that the change in the nucleus-plasma relation takes place along with the change in shape of the cell. This might account for the difference in ratio, but I am inclined to think that there is in reality a change in the nucleus-plasma relation, for this method will give the volume of a parallelopiped whether it be a cube or otherwise.

Also, to check the results obtained in Figure 9, which was made from a frontal section, measurements were made from a sagittal section of a tail at the same time of regeneration. If the entire cell merely spread out, then the section through this plane would certainly not show a decrease in the nucleus-plasma relation, but rather an increase to compensate for the decrease obtained from a frontal section. This, however, is not so. A curve from a measurement of a sagittal section (Fig. 10) shows essentially the same characteristics as the curve in Figure 9. These two curves combine all three dimensions of the migrating cells, whereas a curve from a sagittal or a frontal section alone, will have an unknown

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dimension which has to be estimated. In this way, by measuring and making curves from frontal and sagittal sections, I think I have overcome the objection which might be made because of the estimation of the third or the unknown dimension. The proof of this is that both curves show a decrease in the ratio as they should if there were a true decrease in the cells.

IV. DISCUSSION

As will be evident from the foregoing, the epidermis which covers the wound and the clot comes, without a doubt, from the old epidermis. There is a slight difference in the regeneration in

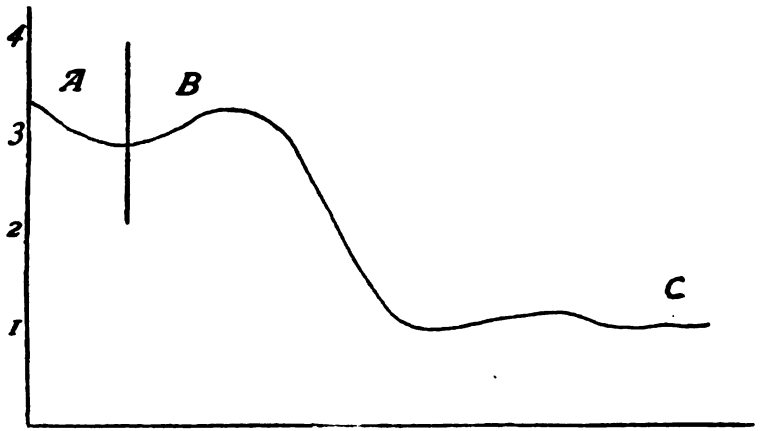

Figure 10

Fig. 10. Curve showing the decrease in the nucleus-plasma relation in the migrating cells, taken from a sagittal section. The abscissa represents the position of the cell in the epidermis, and the corresponding ordinate the nucleus-plasma ratio for the cell. A, region of normal epidermis; B, region of the beginning of migration; C, region of the advancing end and active migration.

this form from that described by Rand (04) for the earthworm, in that he says "as already pointed out, mere contraction of the muscular body wall plays an unimportant part in the closing of the wound, because of the relatively large cross section of the worm." In the tadpole the wound is very narrow except at the region of the notochord, and in one hour's time the contraction of the muscles of the tail is sufficient to cause the meeting of the

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epidermis of the two sides above and below the notochord it was seen that the basal membrane, and therefore the epidermis, even overlaps to a slight degree. So it may be said that the first reparation process, that of muscle contration is of some importance in the early stages of regeneration in this form. There cannot be any doubt that the new epidermis comes from the old because it may be seen in various stages on its way, and because there is no tissue with which it might be confused which has anything like the appearance of the epidermis.

Rand (04) and Emmel (10) differ somewhat as to the internal cell changes. Rand states for the earthworm "the nuclei, however, never show marked change in volume" while Emmel says that in the crustacean "it may be noticed that the nuclei are becoming larger in size, and the chromatin is undergoing certain changes." I could find no evidence in either direction in the tadpole, migrating nuclei being in some cases both larger and smaller than normal nuclei. Rand does not mention any marked chromatin changes, while Emmel describes a kind of "polarization" of the chromatin in the nucleus. I did not find any radical changes in the chromatin except that it seemed to accumulate in larger bodies than normally, and that it was more densely packed, due perhaps to the lengthening of the nucleus.

There can be no doubt that in the tadpole there is a migration of the individual cells of the epidermis. The extraordinary change of shape of the cells, especially those of the outermost layer indicates that the individual cell is moving by lengthening out in an amœboid manner. This migration cannot be otherwise, for if there was a pulling of all the epidermis from the region of the cut surface, all of the cells would take on the elongated form so characteristic of the outermost layer of cells. This, however, is not so, and it is this same outer layer which is the most changed in shape, and which seems to be the most concerned in the process of migration. The deeper cells have the appearance of being pulled along, and to support this hypothesis, instances have been noted, where the deeper layers of cells are obstructed by some of the fragments of the cut tissues, and have been held back, only the outer layer traveling over the obstruction.

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The advancing edge of the migrating epidermis clearly shows then that there is not a passive pushing of the cells over the cut surface. What is the agency by which the cells are stimulated to move over the wound? They always move over the surface. In no case did I find evidences of a migration down into the clot, nor were there any signs of a free migration, that is, without a solid support for the migrating cells. Neither were the advancing cells moving along the fibrils of the clot, as they moved as easily over the cut notochord. The fibrinations of the clot also were too small to give anything more than a rough surface over which the cells might travel. There was nothing like the condition of the spider web which was used by Harrison (14) in his experiments with cell migration. The migration here is more like that shown by the cells which moved along the cover glass in his experiments.

This migration is assigned by the majority of authors to chemotaxis, or a chemotactic cell phenomenon, in the sense of Roux (94) (96). The stimulus may come from the chemical attraction of the wound surface, or the mutual interaction of the epidermal cells. The stimulus starts the epidermal cells moving and as the only direction in which they can move is over the wound surface the effect of their migration is to heal the wound opening. When the two sides meet there is no longer any opportunity for migration as there is no place for the cells to go, so that migration stops. Rand emphasizes the fact that the stimuli operating there are similar in nature to those which are said by Herbst (94) to be of great importance in normal ontogeny.

There have been several references to a change in the size of the cell or nucleus during the migration of the epidermis in various animals. Rand (04) mentions the fact that the cytoplasm of the migrating cells in the earthworm appears to decrease, and Emmel (10) says for the crayfish that the nuclei seem to be slightly larger than normally. Fraisse (85) states that on the advancing edge of the migrating epidermis of the salamander the nucleus is surrounded by a small amount of protoplasm, so that the nuclei are closely packed together. A glance at his Plate I, Fig. 8, shows this feature, and the change in relation of the cytoplasm to the nucleus is plainly to be seen without measurement.

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It seems fairly certain, therefore, that there is a decrease in the nucleus-plasma ration during migration. The measurements given in this paper of course are not useful as actual numerical values of the volumes, being merely to get an approximate curve of the decrease in the ratio value.

Admitting the fact that there is a decrease in the nucleus-plasma ratio, what does it signify? If Minot's hypothesis applies to all forms of cell phenomena in which there is a decrease in the nucleus-plasma ratio, then this might indicate a rejuvenescence of the cell after differentiation. He states "Rejuvenescence depends on the increase of the nucleus" or in terms of the nucleus-plasma relation a decrease in that ratio. This was in connection with cell cleavage of the fertilized egg. He also adds "Reversed cytomorphosis is not known to occur, or in other words differentiated material cannot be restored to the undifferentiated condition." Yet here if the decrease in the nucleus-plasma ratio indicates a rejuvenescence we have differentiated epidermal cells which are rejuvenating and afterward returning to the differentiated condition.

Interesting in this connection is the work of Morgulis (11) on inanition, in which he describes the effect of starvation on the cells of the salamander, Diemycytylis viridescens. He concludes that the volume of both the cell and nucleus decreases as a result of starvation, but that the rate of diminution of the volume of the cells is greater than that of the nuclei.

The basal cells of the epidermis in the tadpole pull away from the basal membrane during migration, and it is possible that this decreases the interchange of nutrient material between the blood and the epidermal cells. Therefore they may be reduced to the condition of starved cells. This, if Morgulis' hypothesis be correct would tend to reduce the nucleus-plasma ratio. Therefore the decrease in the observed nucleus plasma relation may be entirely due to a starvation of the epidermal cells caused by isolation from food during migration.

However, Conklin (12) states that rejuvenescence is dependent on the interchange of material between the nucleus and the cyto-

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plasm, and that anything which facilitates this interchange increases metabolism and leads to rejuvenescence.

The cells are in active motion, and as the supply of food may be cut off, and as they are undergoing an unusual activity the rate of metabolism is without doubt great. This activity is so great that the cytoplasm is probably used up and therefore the nucleus-plasma ratio decreases. Therefore, if Conklin's hypothesis applies to these cells we have a rejuvenescence as there is a high rate of metabolism and consequently an interchange of material between nucleus and cytoplasm.

V. SUMMARY.

In brief the processes which occur in the early stages of regeneration in the tail of Rana clamitans are as follows:

1. There is a muscle contraction within 1 hour after the cut which not only decreases the surface of the wound, but also plays an important part in the process of healing by bringing the epidermis of the two sides of the tail together both above and below the notochord, where the epidermis fuses.

2. There is a migration of the epidermal cells over the clot which is formed at about 12 hours time. This migration is the result of an active migration of the individual cells of the epidermis, especially those of the outer layer, without the slightest sign of cell multiplication of any sort, either by mitotic or amitotic cell division.

3. There is a decrease in the nucleus-plasma ratio in the migrating epidermis.

4. As the cells of the migrating epidermis are pulled away from their normal position in relation to the blood vessels, this decrease may be due to starvation.

5. As there is also a very active migration of the individual cells the rate of metabolism is probably high, and as the cells may not receive their customary amount of nourishment, the cells may be forced to rely upon the energy contained in the cytoplasm, and so use it up during the migration. This would account for the decrease in ratio.

6. If the views of Minot and Conklin can be applied to these cells, then this decrease in the nucleus-plasma ratio would indicate a rejuvenescence of differentiated epidermal cells.

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7. Migration ceases at about 24 hours and the nucleus-plasma ratio returns to normal in the epidermal cells.

8. The period of time from 24 to 48 hours is occupied by the absorption of the clot, and the cells are in a resting condition.

9. At 48 hours regeneration by mitosis begins. No evidence has been found that amitosis is a factor in the regeneration of the tail of the frog tadpole.

This problem was suggested by Dr. Charles Zeleny, to whom I am indebted for many helpful suggestions during the course of the investigation.

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AMERICAN SPECIES OF THE GENUS ATRACTIDES

By Ruth Marshall

The water mites of the genus Atractides belong to a small subfamily, the *Hygrobatinæ*. About ten species have been described by European hydrachnologists for Europe; two of these species have been reported by them also from North America, while one species is described for Madagascar. Three new species are now added to the list for the United States. All of the material, thirty-eight individuals, was found by the author in small lakes in Wisconsin.

The species comprising this group are easily recognized; but, unfortunately, there has been much confusion in the use of the name *Atractides*. By some authors it has been used as a synonym for the genus *Megapus* of Neuman, and given as the valid name for the genus *Torrenticola* of Piersig. The nomenclature used by Piersig (1901) and by Wolcott (1905) seems to the author to be logical, and it is used in this paper.

The body of these mites is oval or elliptical; the integument is usually soft, with a surface covering finely striated or porous. The epimera are in three groups, with the capitulum lying in close contact with the first pair. The most distinctive feature is the oblique placing of the third pair and the more or less truncate margin of the fourth. The genital area is placed about midway between the epimera and the end of the body; the cleft in both sexes is flanked on either side by three conspicuous acetabula on a sickle-shaped plate, another distinctive feature of the genus. In the female the cleft is long and the acetabula are removed from it; in the male the shorter cleft is completely enclosed by the plates bearing the acetabula.

The palpi are about as thick as the legs. The joints of the palpi increase in length from the first to the fourth, while the fifth is small and claw-like. A distinctive feature of the genus is the

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presence of two hairs on papillæ on the ventral side of the fourth joint, and a sword-like spine on the inner side. This fourth joint usually bears some fine hairs.

The legs do not vary greatly in length; all of them bear spines and a few hairs. The first leg is most distinctive; it is often longer and stouter than the second, and the last joint shows more or less bending. These features are pronounced in figures published by European writers on the genus; they are not conspicuous features of the three new American species here described.

Atractides parviscutus nov. spec.

Pl. VI, Fig. 1-4

This is a large mite, the largest specimen measuring 1.3 mm. The epimeral plates occupy an uncommonly small part of the ventral surface. The spaces between the groups of plates are large. The surface of the body, including the plates, is very finely porous. On both dorsal and ventral surfaces the plates marking the openings of the skin glands are prominent; in balsam mounts the glands are strongly outlined, and fine hairs may be made out on the plates.

The legs are much alike; the first two pairs have about the same length. The first pair are only a trifle the stouter, while the last joint shows but little bending. The palpi are slender.

Only the female of the species was found in a collection of twenty-six individuals, all from Wisconsin lakes. Four of these were found in Nagowicka and Neshota lakes, near Milwaukee, in the fall of 1908; eighteen in Lake Spooner, in northwestern Wisconsin, in the summer and fall of 1909; two in Lake Minocqua, in the northern part of the state, in July, 1912; and two in the Lauderdale Lakes, in southern Wisconsin, August 12, 1914.

Atractides phenopleces nov. spec.

Pl. VI, Fig. 5-9

This species is founded upon the examination of a single specimen, a female from Lake Spooner, Wisconsin (Aug. 18, 1909). It measured 1.1 mm. in length. The epimeral plates show fewer of the irregularities of surface than do those of some species, and the

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posterior borders of the fourth plates are sharply truncate. Certain small characters which distinguish this from related species are best brought out by the figures. The lateral plates of the genital area bearing the acetabula are large and somewhat removed from the rest of the area. The first pair of legs show the bending of the last joint so characteristic of the genus.

Atracides orthopes nov. spec.

Pl. VI, Fig. 10-12

This new species is represented here by six males, three from Lake Nagowicka, near Milwaukee (Oct. 15, 1908), and three from Lake Spooner, Wisconsin (July 16, 1909). It resembles *A. gibberipalpus* Piersig in the character of the epimeral plates, which show irregular surfaces and a close proximity of the three groups. The palpi are fairly stout, and the last joint of the first leg is very long and shows no trace of bending; in these particulars the new species differs from the related one. The two sword-like bristles on the distal end of the fifth joint of the first leg are very large. The surface of the body appears very finely granular, the plates more so. The length of the body is 0.65 mm.

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EXPLANATION OF FIGURES

Fig. 1. *Atractides parviscutus* nov. spec., ventral view.

Fig. 2. *Atractides parviscutus* nov. spec., dorsal view.

Fig. 3. *Atractides parviscutus* nov. spec., right palpus, inner side.

Fig. 4. *Atractides parviscutus* nov. spec., legs, left side.

Fig. 5. *Atractides phenopleces* nov. spec., ventral view.

Fig. 6. *Atractides phenopleces* nov. spec., 1st leg, left side.

Fig. 7. *Atractides phenopleces* nov. spec., 4th leg, left side.

Fig. 8. *Atractides phenopleces* nov. spec., genital field, female.

Fig. 9. *Atractides phenopleces* nov. spec., left palpus and capitulum.

Fig. 10. *Atractides orthopes* nov. spec., ventral view.

Fig. 11. *Atractides orthopes* nov. spec., 5th, 6th joints, right 1st leg.

Fig. 12. *Atractides orthopes* nov. spec., right palpus, outer side.

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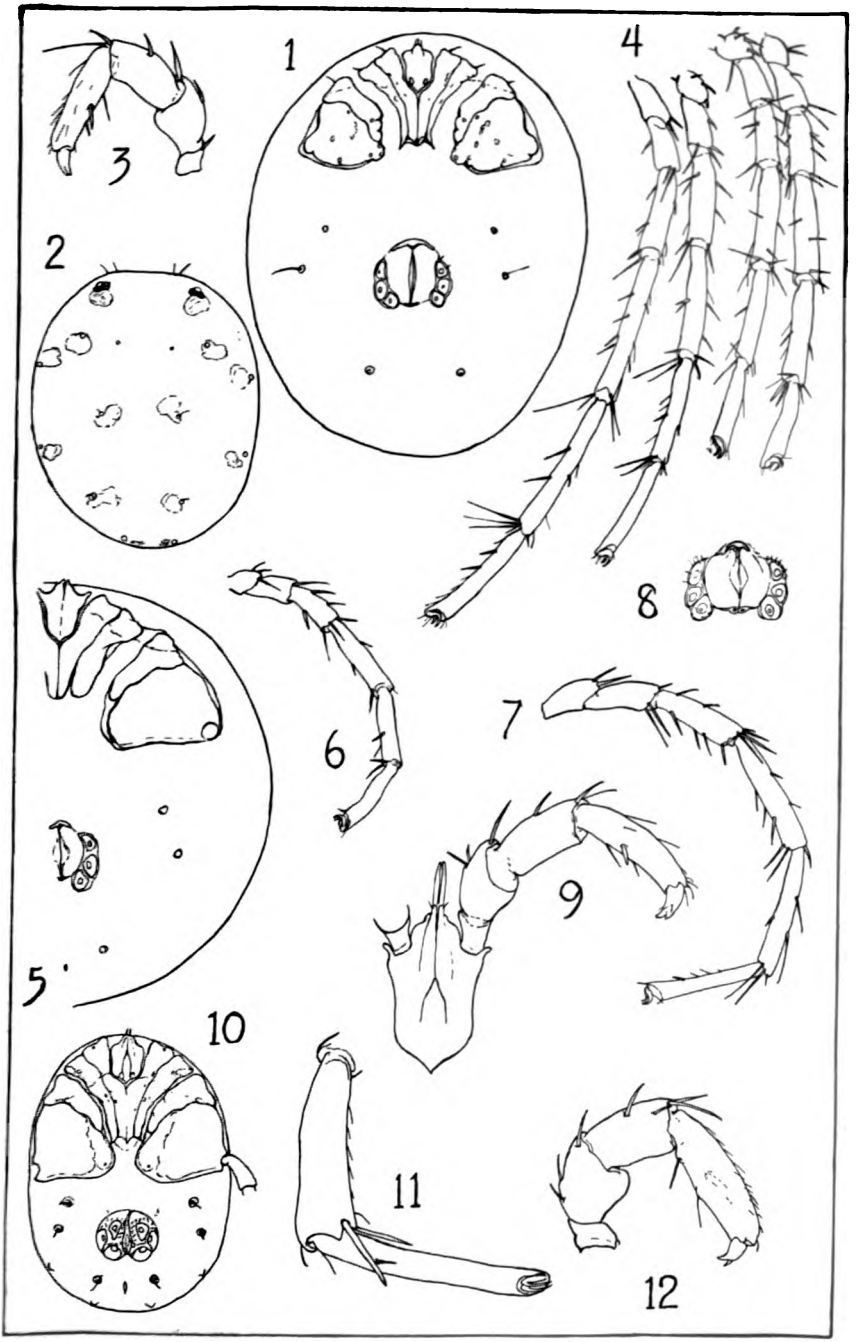

PLATE VI

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DEPARTMENT OF NOTES. REVIEWS. ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

A SYSTEM FOR LOCATING OBJECTS ON MICROSCOPE SLIDES

By N. A. Cobb

All mathematical methods of recording the position of a microscopic object on a slide use a system of coördinates. Most microscope makers provide some scaled mechanism, usually in connection with the mechanical stage, which allows of two readings that locate the position of the object. Sometimes the scales are engraved on the two traverses of the stage, sometimes on a special microscope slide.

The various systems are not in harmony, so that it is often inconvenient to use on one microscope records made on another. There are contrivances for overcoming this inconvenience, some of much merit, but none of them have come into wide use, to say nothing of universal use.

The object of this communication is to propose a basis for agreement so simple that it may possibly win universal support. The proposal is to make one corner of the slide, say the lower left hand corner, the point of origin for the system of coördinates. The lower left hand corner of the slide then becomes zero, and any object is located by telling how far to the right of zero it lies, and how far above zero. Any system of linear measurement may be used.

Thus in the illustration the location of the object "x" is recorded by the figures 41.5-16.5 millimeters; the object is 41.5 millimeters to the right of and 16.5 millimeters above zero.

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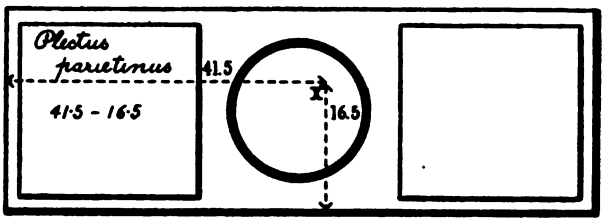
Against certain small objections may be urged a considerable number of practical points. First, the *point of origin is a natural one,* fixed, not registered, that exists on every rectangular slide regardless of its size; in a certain sense therefore it is not arbi-



trary. Second, it is possible to locate objects, approximately at least, *without mechanical apparatus.* This is of importance to a vast number of microscopists who have few accessories. Third, *any point on the slide* is indicated by figures having a positive sign.

To one having a plain simple stage without any other accessories than spring clips it is possible by this system to find the coördinates of an object by the following simple method.

Take a piece of *thin* paper about ten centimeters long by four centimeters wide, or larger, and make a pinhole near the middle. Clamp this paper to the stage with the clips, and bring the pinhole to the center of the microscope field. Place the slide on the paper, turn on a strong light, and it will generally be found that the object can be examined in this fashion. When the object is found, place it centrally in the field, see that the pinhole is also central, and with a sharp pencil point trace the lower and left hand edges of the slide. Finally measure the distances from the pinhole to these two lines. These measurements will be the coördinates desired. To find an object given its coördinates, reverse the process.

ENTOMOLOGICAL NOTES

Stylops and Stylopization.—Smith and Hamm, ('14, Quart. Journ. Micr. Sci., N. Ser., 60: 435-461) find that in spite of the fact that active, winged males of *Stylops* exist, fertilization cannot occur and development is always parthenogenetic. This parasite causes a reduction of about three-fourths in the size of the ovaries of the host bee and no ripe ova are ever produced. The testes are

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usually not affected, and ripe, normal spermatozoa may be formed. The parasitized female bee usually has a reduced scopa and rarely, if ever, collects pollen.

Oögenesis.—Dederer ('15, Journ. Morph., 26: 1-42) presents the results of a study of the oögenesis in *Philosamia cynthia.* Thirteen chromosomes occur in the pronuclei, thus making the somatic number twenty-six. No satisfactory evidence as to the presence or absence of an X-Y pair of chromosomes was secured. No differential divisions were found in the oögonia. The nuclei of the future nurse cells show evidences of preparation for division although the chromosomes disintegrate, forming numerous granules which arrange themselves near the much infolded nuclear wall. The nurse cells contribute material to the egg cell by means of tubes. Amitosis was not observed among the germ cells. During most of the growth period the egg nucleus is in the spireme stage.

Size Dimorphism in Spermatozoa.—Zeleny and Faust ('15, Journ. Exp. Zool., 18:187-240) report the results of a study of size differences in the length of the head of spermatozoa from single testes in fifteen species of animals, of which ten are insects representing five different orders. Long series of measurements yield evidence that the population of spermatozoa is composed of two size groups, due to the difference in chromosomal content. These groups are not wholly distinct. The distribution curve in each species usually showed two high points or modes, and a comparison of the degree of separation of these modes with the expected degree secured by calculations on chromosomal histories shows a significant correspondence.

Termites.—Snyder ('15, U. S. Dept. Agr., Bureau of Ent., Bull. No. 94, Part II) reports the results of a study of the biology of the termites of eastern United States. The scope of the investigation was broad, and important data were secured on communal organization, castes, life cycle, cannibalism, different forms of nest, establishment of new colonies, parasites, termitophilous insects, etc. A few of the important facts revealed by this study are as follows: The life cycle of *Leucotermes* is highly variable. New colonies may be established by three groups of individuals, (1) the sexed colonizing adults which swarm and leave the colony, (2) neoteinic

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royal individuals "produced from nymphs of the second form which never (?) leave the parent colony, or from young larvæ, as in colonies orphaned after the nymphs of the first form have nearly completed their development", (3) neoteinic reproductive forms "supplied to orphaned colonies, which may be derived from nymphs of the first or second forms, or larvæ". A bibliography of about forty titles is given.

Wood-boring Trichoptera.—Lloyd ('15, Psyche, 22:17-21) describes a new species of caddis-fly *(Ganonema nigrum)* from Ithaca, N. Y., the larvæ of which have case-making habits which differ greatly from those of other described American trichopterous larvæ. They bore into the solid wood of twigs, heavy bark, or fragments of broken wood on the stream-bottom and use the tunneled wood as portable cases. The food consists of finely pulverized wood. These larvæ are apparently as active in midwinter when the temperature is 20° Fahr., as in midsummer. The writer has found similar cases in streams in Colombia, S. A. Attention is called to Hudson's record (1904) of a species of similar habits in New Zealand.

Egg Concealment.—Hegner ('15, Psyche, 22:24-27) reports results of experiments on the eggs of the potato beetle *(Leptinotarsa decemlineata)* to determine the reasons why eggs are laid on the lower surface of the leaf rather than the upper. The data show that eggs in various stages of development, if kept in direct sunlight, are not retarded in their development but are prevented from hatching, due, probably to partial desiccation. While the advantage of concealment is, no doubt, important, the advantage secured by protection from the desiccating influence of sunlight is the vital one.

Proventriculus of Hydropsychid Larvæ.—Noyes ('15, Journ. Ent. and Zool., 7:34-44) finds that in the larvæ of the genus *Hydropsyche* (Trichoptera) the well-developed proventriculus is unique in having a large number of distinct comparatively solid, chitinous teeth in the anterior part. Two valves, continuous with the œsophageal valve, occur in the posterior part. The œsophageal valve is open along one entire side. The muscle layers in the fore-intestine do not conform to the usual arrangement in insects but the

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inner layer is composed of circular muscles and the outer layer of longitudinal muscles. The anterior portion of the organ triturates resistant parts of the food while the posterior portion serves the combined function of a strainer and of a regulator of the passage of food into the intestine.

Spider Poison.—Kellogg ('15, Journ. Parasitology, 1:107-112) summarizes literature and presents discussion of the effects of the poison of spiders belonging to the genus *Latrodectes,* the species of which have been regarded as non-venomous or nearly so by some students of the Araneida. Evidence is given in support of the contention that spider poison is formidable and active in character. Allowing for individual differences in physiological condition in different people, it appears that the poison of *Latrodectes* is capable of producing serious results.

Blood-sucking Muscid Larvæ.—Coutant ('15, Journ. Parasitology, 1; 135-150) reports the finding of the larvæ of *Protocalliphora azurea,* one of the flesh-flies, on the bodies of young crows as parasites. The blood-sucking habit of these larvæ was demonstrated by a microscopic examination of the contents of the alimentary canals which were full of the corpuscles of the crow's blood. Data are presented to support the view that the larvæ are intermittent feeders and the author holds that they do not eat solid flesh, either fresh or decayed. The paper includes a discussion of the anatomy of the larvæ and pupa, the habits, distribution, and chætotaxy of the adult.

Morphology of Thysanoptera.—Peterson ('15, Ann. Ent. Soc. Am., 7:20-66) presents the results of a detailed study of the anatomy of the head and mouth parts of the Thysanoptera. The interpretation of the asymmetrical mouth-parts of this order is significant. The results of such an intensive morphological study can not be completely summarized here but the following are a few of the important general points: The asymmetrical mouth-parts of thrips are arranged in the form of a cone and are fitted for sucking. Mandibular and maxillary parts are uniformily present and show asymmetry. The greatly reduced tentorium is homologized and renders important aid in the interpretation of the piercing organs. The piercing organs in the two suborders are homologous. The sucking

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apparatus of the pharynx, the salivary glands and the head glands have been critically studied.

Key to Families of Insects.—Brues and Melander (1915) have published a book entitled "Key to the Families of North American Insects". A brief complete key of the dichotomous type has been constructed and leads to the identification of 483 families. The system of orders adopted is essentially that of Handlirsh, more recently established orders being also included, thus making a total of thirty seven. The chief characters of each order are given. The eighteen plates containing 437 figures of representative insects or their morphological details, the glossary, the index, the pronunciation of the names, and the inclusion of synonyms are useful characters of the book. In spite of the fact that the new system of orders adopted involves a considerable departure from the one commonly used and necessitates a certain amount of readjustment, persons interested in taxonomy of insects will find the book a useful source of information.

New Order of Insects.—Silvestri ('13, Portici Boll. Lab. Zool. Gen. Agr., 7:193-209) describes three species of a very interesting new genus, *Zorotypus,* from the Gold Coast, Ceylon, and Java. A new order, *Zoraptera,* is proposed for the reception of these species, the characteristics being as follows: minute; terrestrial; wingless; agile; predaceous. Body depressed. Antennæ moniliform, nine segments. Eyes vestigial. Mandibles strong. Thorax as long as abdomen. Abdomen with ten segments. Cerci short, conical, one segment. Legs similar; hind femora enlarged; tarsi with two segments. The specimens on which this new order is based are only about 2 mm. long. They occur in rotting, vegetable debris.

Fossil Fungus-Gnat.—Cockerell ('15, Can. Ent., 47:159) describes the first genuine fossil *Mycetophila* found in America. The general appearance and structure are said to be exactly as in the living species, *Mycetophyla punctata* Meigen, but differences in certain details of structure led to the disposal of the form as a new species, described under the name of *Mycetophyla bradenæ.* This fossil was collected from the Miocene shales of Florissant, Colorado. The studies of the European species from Baltic amber of Oligocene agé and the American form show that the fossil forms

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are of the same type as the living ones and it appears that some of the *Mycetophylidæ* have continued through the long lapse of time without evolutionary progress except for the changes in minor specific characters.

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NOTES ON BIOLOGICAL METHODS FROM UNIVERSITY OF ILLINOIS

At the request of the Secretary of the American Microscopical Society the following notes on Biological Methods in use at the University of Illinois have been gathered with an aim toward offering suggestions that might be of value to the readers of the Transactions who are looking for shorter cuts and improved methods in their Biological work. The writer makes no claim of discovery or of originality for the greater part of the methods offered here, in truth many of them are old facts revived while some of them are but modifications of standard biological methods.

For clearing bulky and difficult objects.—The use of synthetic oil of wintergreen as a clearing agent for microscopic mounts of whole objects greatly increases their transparency. Objects which appear opaque after treatment with cedar oil, xylol, or toluol, by the use of synthetic oil of wintergreen become highly translucent. This is especially true in the case of solid parenchymatous animals such as large trematodes, leeches, and planarians. Violent diffusion currents accompanying the transfer from absolute alcohol to the oil may be avoided by running the specimens through a series of mixtures of absolute alcohol and the oil. In most cases three or four such mixtures are sufficient, though for animals with thin body walls and large internal cavities the writer has found as many as six grades necessary at times. From the oil of wintergreen transfer to Canada balsam or damar in xylol on a slide and add coverglass.

Damar as a mounting medium.—Damar dissolved in xylol has many advantages over Canada balsam. Balsam turns a dark brown with age. In mounts of embryos or other objects where considerable volume of the mounting medium is used this change of color soon

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impairs the value of a mount. Damar retains its light color and perfect transparency indefinitely. In preparing either damar or balsam it is well to dissolve in an excess of xylol. This gives a very thin liquid which should be run through filter paper. After filtering evaporate the excess of oil as rapidly as possible to prevent chances of contamination by acid fumes or by dust from the air. This is best accomplished by placing the damar or balsam over a steam bath, though by using care the oil may be driven off by placing the liquid in a porcelain dish over a gas flame.

To facilitate penetration of fluids in organisms with a heavy cuticula.—Many animals covered with an unbroken cuticula strongly resist the penetration of fluids. In many cases where there are large internal cavities this causes the specimens to turn black or sometimes opaque chalky white when brought into the mounting medium, damar or balsam. Some avoid this by cutting the animal into pieces. This allows free penetration through the cut surfaces but is almost sure to lead to confusion in interpreting the relations of parts. The writer has found that several small punctures through the cuticula while the animal is still in alcohol allow the clearing and mounting media to enter freely. By performing this operation under a binocular the holes can be made with a very fine needle in regions of the body where they will least interfere with internal structures.

For best results with the oil immersion objective a drop of immersion oil, or a drop of water, should be placed upon the upper surface of the substage condenser and brought into contact with the bottom of the slide. This prevents refraction of light by the air between the condenser and the slide, thus producing stronger illumination of the field.

Safety razor blades for the microtome.—In using an adapter for safety razor blades in a rotary microtome it has been found that a fairly heavy double-edged blade, such as the Durham-Duplex, gives less vibration and for that reason more uniform and better sections than are possible with the extremely thin blades. The blade mentioned also has a much longer cutting edge.

To hinge the lids of wooden slide boxes.—Lids of the ordinary wooden slide boxes very readily become separated from their prop-

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er boxes and unfortunately are not ofter interchangeable. A strip of gummed cloth tape answers very well as a means of binding these lids and at the same time prevents interchanging the covers.

A table for the microtome.—A suitable table for a microtome that is used by several persons in different rooms often becomes a problem. In the local Department of Zoology this has been solved by using a typewriter cabinet manufactured by the Toledo Metal Furniture Company. This cabinet consists of a table mounted upon wheels. The microtome is bolted to the table. When in use the top is rolled back and the two hinged sides are lowered, forming ample working space. By throwing a lever the stand is raised off the wheels onto a set of legs which gives greater stability. This arrangement serves admirably for ordinary histological work, but for perfect series of thin sections the stand is not rigid enough to give great precision with a rotary microtome.

To remove balsam from old slides and coverglasses.—Balsam may be removed from old slides and coverglasses by boiling them in a strong solution of "Gold Dust". All paraffin should be removed before putting slides to soak in this mixture, otherwise a film which is hard to remove forms over the surface of the glass.

The use of modeling clay in a beginning class.—The difficulty of inducing the beginner with the microscope to interpret miscroscopic objects in three dimensions may be eliminated to great extent by requiring each student to model some animal such as the Paramecium. Modeling clay may be purchased from any artists' supply house.

A transparent seal for museum jars.—Celluloid wet with acetone makes a transparent seal for the covers of museum jars. Celluloid strips, or a ring in the case of a round jar, should be cut just a little wider than the ground glass surfaces to be cemented. The celluloid is then dipped in acetone, placed upon the edge of the jar and the cover applied before the acetone has evaporated. Slight pressure should be applied until the celluloid has set.

A slide marker for use with the microscope.—In dealing with gross structures carbon ink is frequently used to direct attention to a certain section of a series on a slide. Frequently finer details

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need to be marked for future reference. For this work the pen is too uncertain. The Leitz Microscope Company have an object marker which meets the demand for a marker of any sized area admirably. A diamond is fitted eccentrically upon a dummy objective. The spot to be marked is brought into the exact center of the field and the marker is swung into place instead of the objective. By bringing the diamond point into contact with the coverglass and rotating the marker a circle is scratched upon the coverglass around the field desired. The diameter of the circle may be regulated at will by means of a set-screw. In making class demonstrations with the microscope a series of slides once marked may be referred to at any time without the necessity of hunting over an entire collection or even over the slides that are known to contain the demonstrations desired.

An insect mount for class study.—The Department of Entomology has been devising mounts for insects to be used in large classes in economic entomology where it becomes impracticable to furnish each student with several specimens of every form studied. One of the most successful mounts is the one for Lepidoptera which requires for its manufacture: two thin pieces of glass (preferably coverslips for lantern slides), a sheet of heavy cardboard or picture mounting board, and passepartout paper. A square hole of proper size for the specimen to be mounted is cut from the center of the card. An insect either spread or with wings folded may be held in the chamber formed by placing a sheet of glass on each side of the card. The edges of the mount are then bound with passepartout paper. Protection against Dermestidae is secured by cutting one or more holes in the edges of the card and filling these with napthaline crystals.

A double demonstrating eye piece for the microscope.—It is ofter difficult to make sure that the beginning student sees the thing he is supposed to see under the microscope. A double demonstrating eye-piece which fits in the microscope in place of the ordinary ocular enables the instructor and the student to observe the same field at the same time. The adjustable needle pointer in this instrument makes it possible for the instructor to point out the organism to be

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studied and under other circumstances he may require the student actually to point out structures which he thinks he has seen. This instrument is manufactuured by E. Leitz.

Preserving plants in their natural colors.—In the Botany department of this Institution extensive use is being made of a method for preserving green plants in their natural colors. Fifty per cent acetic acid in water is saturated with copper acetate as a stock solution. To one part of the stock solution add four parts of water and in this boil for ten minutes the plants to be preserved. After this treatment put the plants into four percent formalin for preservation. Fungus spots and insect pests show much more naturally in materials prepared in this way than they do in materials that have been bleached out in the preservative.

To seal vials and bottles.—Gutta percha and paraffin melted together give much better results than paraffin alone for sealing corked vials and bottles to prevent evaporation. The gutta percha prevents the mixture from becoming friable.

Ripening hematoxylin.—Many text books on histological methods still hold to the idea that hematoxylin stains must ripen at least three weeks before they are ready for use. By adding a small amount of hydrogen peroxide to a stock bottle of newly prepared hematoxylin the stain becomes usable in a very few hours. The minimum time required for ripening by this process has not been determined though the writer has obtained perfect results from Ehrlich's hematoxylin six hours after it was prepared.

University of Illinois.
Urbana, Ill. H. J. VAN CLEAVE.

SUPERIOR IMMERSION FLUID

For 4 years V. Jensen, (Hospitalstidende, Copenhagen. Dec. 2, LVII. No. 48) has been using a mixture of alpha brom-naphthelin, 24 parts, with 76 parts liquid paraffin, and has found it extremely satisfactory as an immersion fluid. He tests it to get the refraction index the same as that of the ordinary immersion oil. Its advantage over the latter is: It flows easily; keeps perfectly, even uncovered; can be easily removed at any time, early or late; it does not dry out, and the specimen keeps its color perfectly.

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THE LIFE OF THE INLAND WATERS.

Under this title Messrs. Needham and Lloyd are issuing a book that will interest and help beginning students of aquatic biology. It is an untechnical discussion of some of the conditions determining the life of fresh waters, and of the life itself. It will be valuable to many of the members of the American Microscopical Society.

Chapter I treats in an introductory way some of the conditions of aquatic investigation and concludes with a list of the American Stations for the study of Biology in the field. Chapters II and III discuss the nature of water as an environment for organisms, and some of the particular types of fresh water bodies as these determine the life within them. Chapter IV gives an account of the aquatic organisms themselves:—algæ, colorless water plants, moss-worts, fernworts, and seed plants; protozoans, lower invertebrates, arthropods, and vertebrates. Chapter V discusses the individual adjustments of organisms to the conditions of aquatic life and to one another. Chapter VI treats aquatic societies and Chapter VII, inland water-culture.

Aside from its educational value in connection with school courses, the book has interest to the general student because the waters are a source of a considerable part of our food supply, are important in determining the sanitary conditions of our existence on the earth, and are mixed up vitally with our recreation and pleasure.

The Life of Inland Waters. James G. Needham and J. F. Lloyd. Illustrated 1915. Comstock Publishing Co., Ithaca, N. Y.

PHOTOMICROGRAPHY

It can safely be said that the general worker has not yet realized the possibilities of the combination of microscopy and photography in research, in technology, and in industry. In the preparation of the Handbook of Photomicrography, Messrs. Hind and Randles have rendered a real service alike to the general student and to those directly interested in the technique of the photography of microscopic objects.

The following chapter headings will sufficiently indicate the character and scope of the treatment:—Photomicrographic appara-

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tus; The microscope; Objectives and Eyepieces; Condensers; Experiments on illumination; Low power photomicrography; Critical photomicrography; Color screens and color sensitive plates; Exposure; Oblique and dark ground illumination; Opaque objects—metallography; Color photomicrography; Photographic operations; Some applications of photomicrography.

The point of view is primarily that of the photographer, altho there is the description of the theory and technic in both fields. The apparatus is illustrated by the text figures. In addition to these figures there are 44 full-page photomicrographic reproductions illustrating processes discussed in the text. Eight of these plates are 3-color reproductions of direct color-photomicrography. These are remarkably effective illustrations. Each is accompanied by directions as to the method of its production. There are photographs made by low, medium, and high powers; oblique illuminations, dark-ground and opaque objects; instantaneous and time exposures, stereoscopic and by polarized light.

An appendix includes formulae, processes and tables. An index follows. The mechanical appearance of the book is excellent.

Handbook of Photomicrography. Hind and Randles. Pages 292, with 44 plates and 71 text-figures. E. P. Dutton & Co., New York. Price $2.50 net.

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OCTOBER
1915

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Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants
from the Spencer-Toller Research Fund.
(See TRANSACTIONS, July, 1913)

These awards are to stimulate and aid research in Microscopic work

1. **The Grantee must be a member of the Society**
2. **Resulting discoveries must first be offered for publication in the Society Transactions.**
3. **Application should be made to Dr. Henry B. Ward, Chm., Urbana. Ill.**

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TRANSACTIONS

OF THE

American Microscopical Society

VOLUME XXXIV

1915

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This Society holds a Fund, accumulated in honor of these pioneer microscope makers and now amounting to nearly $5,000. The income from this fund is devoted to stimulating research in any field of microscopy.

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NOTICE TO MEMBERS

Business Sessions of the *American Microscopical Society* will be held at Columbus, Ohio, as follows:

Dec. 28, 12:30 P. M., the Executive Committee will lunch together at the Student Union Building, on the Campus of the O. S. U.

Dec. 29, 5 P. M., the annual business meeting. (See program of A. A. A. S. for place).

The Southern Hotel will be the Headquarters of the Society.

T. W. GALLOWAY,
Secretary.

(Address: Beloit College, Beloit, Wisc., after Feb. 1, 1916).

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NORTH AMERICAN FROG LUNG FLUKES

By William Walter Cort*

Trematodes from the lungs of anura were first reported over a century ago by Zeder (1800:160). In 1819 Rudolphi (1819:99, 378) described a lung fluke from *Rana esculenta* under the name *Distomum variegatum*. Altho after Rudolphi a number of workers added to the knowledge of this form, it was not until the work of Looss (1894:71) that an adequate description was given. Later the same author (Looss, 1899:600) proposed for the frog lung fluke a separate genus, *Hæmatolœchus*, and upon re-examination of his old *Distomum variegatum* material found that it included three distinct species. The largest of these he designated the type of the genus under the name *H. variegatus* and the others he named *H. similis* and *H. asper*. Stiles and Hassall (1902:20) called attention to the fact that in their opinion Looss (1899:602) in mentioning the name *Distoma simile* in connection with his preparations had given this name to the second of these species before the name *Hæmatolœchus similis*. The name *Distoma simile* which is preempted they call a "still born" homonym and propose for the species the combination *Hæmatolœchus similigenus*. Since the only name proposed by Looss was *Hæmatolœchus similis* this specific name in my opinion should not be superceded. *Hæm-*

*Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 53.

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atolœchus asper Looss labels as a species inquirenda since he had only two specimens, but later work has placed the standing of this species beyond any doubt. In 1902 on account of Stäl's hemipteron genus *Hæmatolœcha* established in 1874, Looss (1902:732) changed the generic name *Hæmatolœchus* to *Pneumonœces*. He did this influenced by Braun's (1901:55) contention that if family or subfamily names were formed from generic names which differed only in ending, they would be identical. This seems to me to be a logical application of the rule of priority and I shall accept the later name *Pneumonœces*.

Leidy (1851:207; 1856:44) was the first to notice lung flukes in American frogs, but his descriptions are too brief to make precise diagnosis possible. In 1902 Stafford (1902:895) described five new species of this genus from Canada, and in a later publication (1905:687) he designated them *Pneumonœces longiplexus, P. breviplexus, P. varioplexus, P. similiplexus* and *P. medioplexus*. In regard to *P. varioplexus* Stafford (1902:906) writes:

"I have obtained it in Toronto and Montreal, but I find that I have only few mounted specimens and imperfect notes; consequently I shall not describe it at length but shall trust to the drawing to illustrate its chief characters. I shall be glad to come upon it in numbers for greater assurance as to its claims for specific distinction."

It has not been possible to obtain from Stafford this or any other of his type specimens for examination. Except in the size of the eggs the characters of the above species agree with those given for *P. similiplexus*. I have never collected any lung flukes which might be assigned to this species and at present would feel inclined to consider its distinctness as very doubtful. I shall therefore treat it as a species inquirenda. Pratt's (1903:29) *Ostiolum formosum* Stafford (1905:687) considered to be a synonym of *Pneumonœces medioplexus*. A study of Pratt's type material has confirmed this view. The only other account of frog lung flukes from North America is Seeley's (1906:248) description of *P. complexus*, from *Rana pipiens* in North Carolina. The present paper adds a new species found in Colorado, for which the name *Pneumonœces coloradensis* is proposed. Two other species of

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the genus have been described, one by Klein (1905:60) from India as *P.* capyristes and the other by S. J. Johnston (1912:320) as *P. australis* from Australia.

Work on the trematodes of North American frogs was undertaken by the writer in the fall of 1909 while a graduate student at the University of Illinois. Two previous publications (Cort 1912, 1913) have covered part of this work. In addition to the studies on the lung flukes at that time collection and observations have been made of these forms from a number of different localities. Material has been studied of *Pneumonœces longiplexus, P. breviplexus, P. similiplexus, P. medioplexus, P. complexus* and of the new species from Colorado, *P. coloradensis.*

I wish to express my appreciation to Professor Henry B. Ward for his guidance during the early part of this work, for the use of his library and for material from his private collections. Thanks are due Dr. George R. La Rue for help in tecnic, and to Dr. H. S. Pratt, Mr. Hermann Douthitt and Dr. M. C. Hall for material.

Tecnic

The worms for sectioning were usually killed after shaking according to Looss' method (Looss, 1901) in saturated corrosive sublimate plus 1 to 3% acetic acid. The specimens which were destined for toto mounts were usually transferred into distilled water before killing, in order that part of the eggs might be evacuated. After this treatment they were killed without shaking. All specimens were left in cold killing fluid from 6 to 18 hours, but when hot fluid was used less time was required. For toto staining Mayer's Paracarmine, Mayer's Hæmalum and Delafield's Hæmatoxylin were found most useful. The worms were much overstained in dilute solutions and then very rapidly and completely destained in 2 to 4% HCl in 70 to 85% alcohol. This method of differentiation has been found useful for most toto mounts of trematodes, since in this way almost all of the parenchymatous tissue is cleared of stain, so that the internal organs stand out clearly. For staining sections hæmatoxylin with eosin as a counter was my favorite combination. Much time can be saved by

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staining the worms in toto in Ehrlich's Acid Hæmatoxylin, differentiating on the slide after sectioning and then counterstaining in the higher grades of alcohol. By this method it is not necessary to run the sections on the slide thru a lower grade of alcohol than 85%. The only difficulty in the tecnic of the frog lung flukes is due to the presence of great masses of eggs. In some of the largest specimens sectioning became almost impossible on account of these great masses of eggs. When possible all species were studied alive.

General Discussion

Occurrence. Altho reports of the occurrence of North American frog lung flukes cover an area including Canada and the north eastern and central portions of the United States, they are relatively so few and scattered that little idea *can* be gained of the range of the genus and the distribution of the several species.

Host. All reports of North American representatives of the genus *Pneumonœces* are from *Bufo* or *Rana*. There seem however to have been few if any examinations of the smaller anura. A given host may harbor even in a restricted locality several species, and one species may infest several different hosts. *Pneumonœces longiplexus* and *P. breviplexus* have been found in *Rana catesbiana; Pneumonœces longiplexus, P. similiplexus, P. medioplexus, P. complexus* and *P. coloradensis* in *Rana pipiens;* and *Pneumonœces medioplexus* and *P. breviplexus* in *Bufo americanus*. In several specimens of *Rana pipiens* which were examined from near Chicago there were found represented three species, *Pneumonœces medioplexus, P. similiplexus,* and *P. longiplexus,* in several cases individuals of two different species occurring in the same frog and even in the same lung. Stafford found *P. longiplexus* to be the most common species in *Rana catesbiana*. In my collections from *Rana pipiens, Pneumonœces medioplexus* is by far the commonest form. All degrees of infection have been found, varying with the locality from a few flukes in a small proportion of the frogs examined to a heavy infection in a large proportion. The heaviest infection of frog lung flukes ever reported was in a specimen of *Rana pipiens* from Oshkosh, Wisconsin. This frog contain-

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ed twenty full grown specimens of *Pneumonœces medioplexus* in one lung and twenty-two in the other. The parasites almost filled the lung cavity, their actual bulk being greater than that of the lung tissue.

SIZE. Within the limits of a given species there is found great variation in the size of sexually ripe individuals, i. e. individuals in which the uterus up to the genital pore contains fully developed eggs. In those species in which a considerable number of individuals were available for study, the largest sexually ripe forms were two and even three times the size of the smallest. Between the extremes were all gradations. Some difference in size seems to be due to differences in environment. The average size of the specimens of *P. longiplexus* which I have collected from *Rana pipiens* is considerably less than that of those collected by Stafford from *Rana catesbiana*. From a region in northern Minnesota all the specimens of *Pneumonœces similiplexus* found, altho crowded with eggs were considerably below the average size for the species. In cases however where different sizes were found in the same locality or host, it seems probable, as Stafford (1902:-896) suggests, that these flukes continue to grow in size after they have reached sexual maturity. Such variations in size within a species make any statements of average size either for the whole animal or for its organs of little value. Suckers and reproductive organs usually vary in size in proportion to the size of the individual, only the size of the eggs and the spines remaining constant. For this reason measurements will always be given for individuals, and mere size comparisons, except in the case of the eggs and spines, will be given little weight in specific diagnosis. However ratios in size such as between oral suckers and acetabulum, and ovary and testes, are found to be quite constant within the species and give some of the most important criteria for specific determination.

SPINES. Great variation in regard to the spines is found among the species of this genus (Fig. 4). Some species are entirely covered with them, some are only partially covered, and in some of the cuticula is entirely smooth. It would seem from the general small size of the spines and their complete absence in

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certain species that in the genus *Pneumonœces* they are of little value to the individual and are in the process of degeneration. That there may be variations in this character within a species is suggested by the difference in Stafford's and my own account in regard to the presence and absence of spines in *P. longiplexus* and *P. breviplexus* (see specific accounts). In the individuals which I have examined there have been no variations within a species in the character of the spines.

Suckers. The acetabulum is always smaller than the oral sucker; in several species it shows degeneration and is apparently of no functional value to the adult.

Digestive System. The characters of the digestive system appear to be of generic value and show specific difference of value for diagnosis only in the ratio in size of the oral sucker to the pharynx and in the length of the esophagus, altho this character is almost entirely obscured by differences in the contraction state of the anterior ends of different individuals.

Male Reproductive System. Variations in the size, shape and position of the testes give one of the easiest ways of identifying the species of the frog lung flukes (Figs. 2, 3, 8, 9 and 10). The testes are located diagonally in the body usually back of the middle, with the posterior testis on the same side as the ovary. From the anterior tips of the testes pass forward the vasa efferentia which extend near the dorsal surface of the body forward on each side of the seminal receptacle and unite just in front of the ovary to form the seminal vesicle. The seminal vesicle is a long narrow sac filled with sperms and enclosed for its entire length in a thin walled cirrus sac. Just back of the genital pore the seminal vesicle narrows into an ejaculatory duct, surrounded by a group of unicellular prostate glands, and a short cirrus with thick walls. The cirrus varies with the species from sausage to pear shaped. Both the ejaculatory duct and the cirrus are enclosed in the cirrus sac. When the cirrus is not protruded the male passage opens with the metraterm at the common genital pore. Figures 11 and 12 show the relations of these parts for two of the species. In one specimen of *P. medioplexus* which had been placed in distilled water, the cirrus was extruded and a quantity of sperms ejected. The

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length of the extended cirrus was equal to about one-half the width of the body at this region. In one toto preparation of *P. longiplexus* the cavity of the cirrus contained ripe eggs. This shows a continuity of passage between the metraterm and the cirrus, and suggests the possibility of self-fertilization. This case is parallel to the one Looss (1894:61, Taf. VI, Fig. 127) notes for *Distomum cygnoides.*

Female Reproductive Organs. The general position of the ovary and the ducts near it are characters of generic rank. Specific differences are to be found in the size and shape of the ovary and slight variations in the size and position of the ducts. The ovary which is rounded, oval, or irregularly lobed, according to the species, lies to one side of the middle of the body just back of the acetabulum. In all the species the condition of sexual amphitypy is found, the ovary being sometimes on one side of the body and sometimes on the other, and the other genital organs in like relation. Lying in the midline of the body and dorsad of the ovary, covering a considerable area, is a group of unicellular glands, the so-called "shell gland." This gland surrounds the female ducts of this region. After reading Goldschmidt's (1909) paper on the yolk and shell glands of trematodes, in which he ascribes the shell producing function not to the so-called "shell gland" but to the vitelline glands, I was struck by the absolute inadequacy of such a gland as the "shell gland" of the frog lung flukes to furnish the material for the shells of the great numbers of thick shelled brown eggs which distend the uteri in these forms. I agree with Goldschmidt that this gland cannot be the shell gland and will follow his usage in calling it Mehlis' gland. In the ducts of the vitellaria could be distinguished the same shell droplets described by Goldschmidt. These facts force me to the conclusion that in the frog lung flukes as well as in the forms described by Goldschmidt the vitelline glands produce the shell material. The seminal receptacle is round or oval in shape near the mid line of the body ventrad of the ovary and Mehlis' gland and overlapping the posterior portions of both. It is a thin walled sac as large or larger than the ovary and is full of disintegrating sperm cells in the larger specimens. No Laurer's canal is present. The oviduct starts as

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a narrow tube from a point on the median dorsal side of the ovary. It passes immediately into Mehlis' gland and receives the duct from the seminal receptacle, which is very short and passes up from the dorsal side of that organ. Just after having been joined by this duct the oviduct is met by the medium duct of the vitellaria from the vitelline reservoir. It then passes thru Mehlis' gland as the oötype which is recognizable by its heavy walls and becomes the beginning of the uterus. The uterus runs forward a ways along the inside of the ovary before it turns backward in its course. The vitellaria are much divided, extending for a considerable distance along each side of the body near the dorsal body wall. At either or both ends there are median groups of acini. The groups are composed of numbers of small acini and are connected by longitudinal ducts. These are gathered from each side just back of the ovary into two median ducts, which join just back of the ovary into the median vitelline reservoir (Figs. 13 and 14) *VD, VR.*) While there is much individual variation in the number and arrangement of the groups of acini and the ducts, each species is constant within certain limits. Also the size of the acini and the number in a group vary with the species.

Uterus. Differences in the arrangement of the uterine coils give a character by which certain species can be readily distinguished. The general course of the uterus is from the ovary to the posterior end and back to the genital pore, filling especially in the larger specimens all the available space between the organs. There may or may not be longitudinal folds from the posterior end outside of the intestinal ceca (Figs. 2, 3, 8, 9, 10). Three of the American species have no lateral longitudinal folds of the uterus outside of the intestinal ceca. These forms agree in having a narrow post-acetabular region, so that the genital organs force the intestinal ceca close to the sides of the body. Those species in which lateral longitudinal folds are present have a distinctly widened posterior body and considerable space between the reproductive organs and the lateral margins. *P. longiplexus* in which these folds reach furthest forward has a body distinctly widened clear up to the pharynx. Since in their development the folds of the uterus force their way into all available space between the

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organs, the course of the folds would depend to a considerable extent on the size of the body and the size and shape of the organs. In the development of the narrow forms there is no space outside of the intestinal ceca for the posterior folds of the uterus to grow into, altho one very large specimen of *P. medioplexus* showed contrary to the usual condition in this species short folds outside the intestinal ceca.

Eggs. The uteri in these forms are crowded throughout their lengths with enormous numbers of small eggs. When fully developed they are dark brown and thick shelled and when evacuated contain an embryo in a very early stage of development. Altho in some species the largest eggs may be as large as the smallest or even the average for another species, the average sizes of large numbers of eggs for each species show very distinct differences (Fig. 1, *A, B, C, D, E, F*). In giving the egg size for a species both the range of variation and the average will be recorded. A considerable deviation in the egg size for the species *P. similiplexus* was found in the eggs of three specimens from a single specimen of *Rana pipiens* from Oshkosh, Wisconsin. This variation which has been already noted in another connection (Cort, 1915) is of interest in showing that there may be variations within a species in a character which has up to this time proved so universally constant.

Life-History. Practically nothing is known of the life-history of the frog lung flukes. No account is to be found in the literature of the intermediate host and the development of the cercariæ. Ssinitzin (1907:34) found stages of *P. variegatus* free in the body cavity of both nymphs and adults of the damsel-fly, *Calopteryx virgo*. By feeding experiments he was able to infect frogs with these forms, showing that the insect here serves as the transfer or secondary intermediate host. Stafford (1902) gives some data on immature forms in frog lungs but makes no attempt to follow out the development of organs. For the species *P. coloradensis* I obtained from a frog's lung a considerable series of different ages which will be considered in connection with the description of that species. A very immature specimen (Fig. 5) suggests from the character of its excretory and digestive systems that the cercariæ of the lung flukes belong to the Xiphidiocercariæ.

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Systematic Section

Pneumonœces Looss 1902

Syn. *Hœmatolœchus* Looss 1899, preoccupied

Diagnosis. Body flattened, elongate, tapering anteriorly; cuticula delicate, wholly or partially covered with spines, or smooth; acetabulum smaller than oral sucker, sometimes very small and of little functional value; digestive system with large oral sucker, no prepharynx, good sized pharynx, short esophagus and long intestinal ceca extending to the posterior end of the body; excretory vesicle Y shaped, with long median stem extending up to the region of the ovary and short branches; genital pore in mid-ventral line just back of the oral sucker; long cirrus sac extending from the acetabulum to the genital pore and enclosing a long seminal vesicle, a short ductus ejaculatorius surrounded by prostate glands, and a short protrusible cirrus; ovary with or without lobes just back of the acetabulum; testes round, oval to elongate, with or without lobes, on opposite sides of the body, back of the ovary; Laurer's canal absent; large seminal receptacle present; vitellaria divided into groups of small acini arranged along the sides of the body from in front of the acetabulum to back of the posterior testis; uterus much coiled extending to the posterior end of the body, in some species with longitudinal folds outside the intestinal ceca; eggs very numerous, small with dark brown shells; habitat in the lungs of Anura.

Pneumonœces longiplexus Stafford 1905

Syn. *Hœmatolœchus longiplexus* Stafford 1902

Diagnosis. Characters of genus; large thick species; cuticula covered in all parts of the body with a dense coating of small spines; oral sucker twice as large as acetabulum; ovary somewhat lobed; testes long and narrow, the posterior extending almost to the posterior end of the body; cirrus pear-shaped; vitellaria of very small acini, with from 16 to 26 in a group; uterus with longitudinal folds outside the intestinal ceca extending to the pharynx; eggs average 0.0248 mm. in length and 0.015 mm. in width; from lungs of *Rana catesbiana* and *R. pipiens*.

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DESCRIPTION. *Pneumonœces longiplexus* was found by Stafford (1902:901) to be very common in *Rana catesbiana* Shaw, in Ontario, Quebec, New Brunswick, and Nova Scotia. I have found this species infrequently in *R. pipiens* from near Chicago, Illinois. In several hundred frogs from this region it occurred to the number of fifteen specimens in ten hosts. Also four specimens of this species were found in a bull-frog, *R. catesbiana,* caught near Urbana, Illinois, and others from the same host from North Judson, Indiana.

The specimens of *Pneumonœces longiplexus* from *Rana pipiens* are on the average smaller than those described by Stafford from the bull-frog. Their position in the frog's lung was different from that of any of the other lung flukes found. Instead of hanging by the oral sucker they were coiled up among the folds of the lining of the lung and pushed against the wall so that they were visible externally. As soon as the infected lung was opened under saline solution, they floated away without the tearing of tissue necessary in the loosening of the other species. The freed worms showed considerable power of movement but no locomotion. The pre-acetabular region especially was capable of extension into a long slender neck which was swayed backward and forward.

Well extended toto mounts of this worm are in the form of an elongate ellipse (Fig. 10) with the anterior end bluntly narrowed. The smallest of the mounted specimens was much distended with eggs and had a length of 2.64 mm. and a width of 1.17 mm. An averaged sized mount was 4.6 mm. long and 2 mm. in width. Stafford gives the size of mounted specimens of this species from the bull-frog as 7 to 8 mm. in length and 2 mm. in width. He also notes one living specimen which measured up to 15 mm. in length. This species had a thickness greater than half its width. In one series cross sections measured in the pre-acetabular region 0.93 mm. in width and 0.59 mm. in thickness, thru the ovary 0.96 mm. in width and 0.54 mm. in thickness, and thru the middle of the testicular region 0.86 mm. in width and 0.43 mm. in thickness.

The cuticula in *Pneumonœces longiplexus* for the specimens which I have examined is 0.008 to 0.01 mm. in thickness and is

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covered in all regions of the body with a very dense coating of spines, about 0.001 to 0.002 mm. apart and 0.004 to 0.006 mm. in length. The spines point backward and are set only part way thru the cuticula (Fig. 4, *A*). Stafford's account of the cuticula in this species is entirely different from the description given above. He writes (1902:902) of the cuticula of *P. longiplexus*:

"The cuticle in this species is thick and perfectly smooth, there being no trace of spines either in the fresh worms or the preserved sections." Whether this difference is due to a variation within the species or an error in Stafford's account is impossible at the present writing to determine.

The ratio in size in this species of the oral sucker to the pharynx varies from 2:1 to 5:3, and that of the oral sucker to the acetabulum from 5:2 to 2:1. In a large mount 7 mm. in length the oral sucker was 0.36 mm. in length and 0.42 mm. in width, the pharynx 0.22 mm. in length by 0.18 mm. in width and the acetabulum 0.17 mm. in diameter.

In *P. longiplexus* the ovary is just back of the limits of the first third of the body length, and on account of the extreme length of the testes the reproductive field fills practically all of the posterior two-thirds of the worm (Fig. 10) The testes (Fig. 10, *T*) are very characteristic. They are much elongated, and extend almost to the ends of the intestinal ceca, with the one on the same side as the ovary reaching slightly posteriad and being longer than the other. They are irregular cylinders tapering to both ends and more than twice as long as wide. At their anterior ends they taper for about two-fifths of their lengths to the tip from which the vasa efferentia are given off, while at the posterior end they are bluntly pointed. In a mount 4.4 mm. long the posterior testis extended posteriad 0.4 mm. farther than the anterior, within 0.54 mm. of the posterior end of the animal. The posterior testis of this same specimen was 1.26 mm. long by 0.32 mm. wide and the anterior was 1.08 mm. in length and 0.27 mm. in width. In a cross section 0.9 mm. wide by 0.54 mm. thick thru about the middle region of the testes of another worm, the posterior testis measured 0.23 mm. in width and 0.43 mm. in thickness and the anterior 0.20 mm. in width by 0.36 mm. in thickness.

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The male ducts show characteristics of specific value in the cirrus which when not protruded is a short thick pear-shaped organ, and in the shortness of the ejaculatory duct (Fig. 12, *C*).

The ovary (Fig. 10, *O*) is a somewhat oblong body irregularly lobed, lying a little to one side of the middle of the worm, with its anterior end dorsad of the acetabulum. In a mount 6.8 mm. long the ovary was situated 2.2 mm. from the anterior end with its long axis slightly diagonal to the long axis of the animal, and measured 0.85 mm. in length and 0.72 mm. in width. It is not as thick as in the other species of lung flukes studied. In a cross section thru its middle measuring 1.3 mm. in width and 0.63 mm. in thickness the ovary measured 0.65 mm. in width by 0.22 mm. in thickness.

Lying a little behind and ventrad of the ovary in almost the exact center of the animal is the seminal receptacle (Fig. 10, *SR*), a regularly oval organ, slightly elongated with its long axis coinciding with the long axis of the worm. It is slightly larger than the ovary and fills the space between it and the ventral body wall, overlapping it for about three-fourths of its length. In a frontal section thru the middle of a specimen 3.6 mm. in length the seminal receptacle measured 0.51 mm. in length by 0.38 mm. in width, and in a transverse section 0.98 mm. in width by 0.37 mm. in thickness it measured 0.32 mm. in width by 0.27 mm. in thickness. The connections of the female reproductive organs of *P. longiplexus* are shown in figure 14.

The vitellaria (Fig. 10, *Y*) of this form are characterized by the large number of acini in a group, from 16 to 26, and by the small size of the individual acini, which in a specimen of medium size varied from 0.064 mm. to 0.096 mm. in length and from 0.040 to 0.064 mm. in width. The vitellaria extend from a point about half way between the anterior tip and the acetabulum to within a short distance of the posterior extremity. There was to be found considerable individual variation but this could not be worked out in detail, because in most of the specimens available the vitellaria were hidden by great masses of eggs. Figure 10 gives an idea of their arrangement in one specimen.

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The uterus (Fig. 10, *U*) passes forward from the oötype around the anterior end of the ovary and then thru the region back of this organ in a series of transverse folds. From just in front of the testes it passes directly back between these organs to the posterior end of the animal, where it makes two very long and voluminous longitudinal folds outside of the intestinal ceca up to the region of the pharynx. From the posterior end of the animal it then passes forward in a series of median coils, more or less voluminous depending on the quantity of eggs present, which are ventral in position and for the most part transverse in direction. Toward its anterior end it narrows into a metraterm about 0.45 mm. in length (Fig. 12, *M*). The uterus is characterized by its large caliber, the simpleness of its folding by the great length of the lateral longitudinal coils outside the intestinal ceca and by the general longitudinal direction of its coils.

Fully developed eggs in this species (Fig. 1, *E*) vary from 0.022 to 0.027 mm. in length and from 0.014 to 0.017 mm. in width. The average of a large number of counts from several different individuals was 0.0248 mm. in length by 0.015 mm. in width.

The above account supplements Stafford's original description of the species. Differences were found in the size of the animals and the presence of spines in my specimens.

Pneumonœces breviplexus Stafford 1905

Syn. *Hæmatolœchus breviplexus* Stafford 1902

Diagnosis. Characters of the genus; largest American frog lung fluke; thickness almost half width; cuticula thick, entirely smooth; oral sucker twice the size of acetabulum; ovary very deeply lobed; testes elongate, usually lobed; acini of vitellaria 12 to 20 in a group; longitudinal folds of uterus outside intestinal ceca reaching in front of posterior testis; eggs average 0.0225 mm. in length by 0.0144 mm. in width; from lungs of *Rana clamitans, R. catesbiana* and *Bufo americanus.*

Description. Stafford (1902:904) described *P. breviplexus* from Canada from the lungs of the bull-frog, *Rana catesbiana.* He found this species not nearly so common in this host as *P. longiplexus* and sometimes in the same host as that species. The first

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specimens of this species which came to my notice were from the common toad, *Bufo americanus,* from Oklahoma. Later several were taken from the lungs of specimens of *Rana catesbiana* and *R. clamitans* from North Judson, Indiana.

This is the largest American representative of this genus, being approached only by *Pneumonœces longiplexus.* The largest mount which I have is of one of the specimens from *Bufo americanus* which in a somewhat contracted condition measures 9.4 mm. in length and 2.74 mm. in width. In a normal state of contraction the body is spindle shaped, tapering slightly to the blunt posterior end and with the pre-acetabular region somewhat more tapering (Fig. 9). The smallest specimen of this species examined had the uterus in complicated folds crowded with eggs. It had a length of 5.8 mm. and a width of 1.3 mm. in the region of the anterior testis. This mount was used in making the drawing for this species since the structures were less obscured by the uterine coils than in the other specimens mounted. The thickness was about equal to half the width. In a series of cross sections the width at the region half way between the anterior end and the acetabulum was 1.1 mm. and the thickness 0.7 mm.; thru the ovary the width was 1.7 mm. and the thickness 0.85 mm.; and in the region of the anterior testis the width was 1.8 mm. and the thickness 0.7 mm.

The ratio of the oral sucker to both the pharynx and the acetabulum averages about 2:1. The actual size of these structures is of little value in specific diagnosis since they are subject to so much individual variation. In a specimen 9.4 mm. long by 1.4 mm. wide the oral sucker had a transverse diameter of 0.28 mm. and the pharynx 0.145 mm. while in the smallest specimen studied which had a length of 5.8 mm. the oral sucker had a transverse diameter of 0.4 mm. and the pharynx of 0.19 mm. The ratio was found in all cases to vary but slightly and the size usually varied directly with the size of the individuals.

The cuticula in all the specimens of this species which I have examined is very thick and entirely without spines. In a place where there is an average extension the cuticula is from 0.25 to 0.30 mm. in thickness. Stafford (1902:904) writes of the cuticula and spines of this species:

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"The cuticule of this species is thick, but unlike the preceding species (*P. longiplexus*) is beset with numerous backward projecting spines." Either the presence of spines is a variable factor or Stafford confused his observations.

In *P. breviplexus* the genital field, without the vitellaria takes up about half the length of the body and extends to within about one-fifth of the body length of the posterior end. The ovary (Fig. 9, *O*) is to one side of the median line and is very deeply lobed on its lateral margin. This deep lobing of the ovary is in marked contrast with the other species of the genus in which this organ is unlobed or very slightly lobed. The seminal receptacle is an unlobed structure ventrad of the ovary and about the same size as that organ. The testes (Fig. 9, *T*) are elongate structures irregularly lobed. Sometimes the lobing is along the median margin and sometimes along the lateral margins. The testes overlap for a part of their length. In the specimen 9.4 mm. long and 2.74 mm. wide the ovary had a length of 1.57 mm. and a width of 0.92 mm. The testes overlapped for the distance of 1 mm. and the posterior extended within 2 mm. of the posterior end. The anterior had a length of 1.71 mm. and a width of 1.03 mm. and the posterior had a length of 2.31 mm. and a width of 1.1 mm.

The vitellaria in this species (Fig. 9 *V*) are very like those of *P. longiplexus*. In a specimen of average size the acini measure 0.08 mm. to 0.12 mm. in length and 0.056 mm. to 0.096 mm. in width. Since the size of the vitellaria varies with the size of the animals these measurements are only of value in broad general comparisons. The number of acini in a group varies from 12 to 20 with an average number of 15 of 16. In a few specimens in which all the vitellaria could be made out the number of groups varied from 16 to 20. The number of individuals studied were too few to make any statement of individual variations or average conditions for these structures of much value. However the general condition shown in Figure 9 held for the five individuals in which these structures could be made out.

The folds of the uterus in *P. breviplexus* are large, as in the previous species. From the oötype the uterus passes posteriad between the testes to the posterior end. From here long longitudi-

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nal folds extend outside the intestinal ceca. There is considerable variation in the length of these folds apparently correlated with the number of eggs present. In the individuals where they were shortest they extended in front of the posterior testis and in one case where the worm was much distended with eggs the fold on the same side as the ovary extended to the middle of that structure and the one on the other side in front of its anterior margin. This approaches rather closely to the condition found in *P. longiplexus*. Stafford must have made his description of this species from a few individuals since he describes the longitudinal folds of the uterus as short and from this character picks the specific name *breviplexus*. From the posterior end the ascending part of the uterus coils forward filling the available space between the ovary and the testes. In front of the ovary it passes forward in short transverse folds up to the genital pore, in very large specimens forming a black mass in this region.

The uterus is for its whole length crowded with very small eggs (Fig. 1, *F*). Measurements of fully developed perfect eggs give a variation in length from 0.0205 mm. to 0.026 mm. and in width from 0.013 mm. to 0.016 mm. with an average length of 0.0225 mm. and a width of 0.0144 mm. This is the smallest egg size recorded for any of the species of *Pneumonœces*.

P. longiplexus and *P. breviplexus* are very closely related. They are the largest and heaviest of the frog lung flukes. Both show lobing of the ovary, altho that of *P. breviplexus* is much more pronounced, and both have elongate testes. The vitellaria of both show similar arrangement and have small numerous acini in the groups. Further the lateral longitudinal folds of the uterus outside the intestinal ceca are longer in these two species than in any others of the genus and the eggs are very nearly the same size, being the smallest for the genus. Slight differences between the species show in almost every part. The most useful for distinguishing them are the differences found in body shape, length of the lateral longitudinal folds of the uterus and shape of the ovary and testes.

Pneumonœces similiplexus Stafford 1905

Syn. *Hœmatolœchus similiplexus* Stafford 1902

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DIAGNOSIS. Characters of genus; cuticula with scattered spines which thin out back of acetabulum and are entirely absent back of the posterior testis; ratio of oral sucker to acetabulum 4:3; ovary and testes without lobes, small round or oval; acini of vitellaria large with 6 to 13 in a group; longitudinal folds of uterus outside of intestinal ceca reaching beyond posterior testis; eggs average 0.0376 mm. in length by 0.0184 mm. in width; in lungs of *Rana pipiens* and *Bufo americanus*.

DESCRIPTION. The original description of *Pneumonœces simíliplexus* is by Stafford (1902:907). He reports this species from Canada from the lungs *Rana virescens* Kalm and from *Bufo lentiginosus* Shaw. I have found it only in *Rana pipiens*, sometimes occurring with *P. medioplexus*, but never as common as that form. This fluke (Fig. 3) is spindle shaped, widest just back of the middle of the body and tapering gradually toward both ends, with the pre-acetabular region somewhat long and narrow. Stafford notes that this species is more inclined to be cylindrical than the other species he studied of this genus. However, all the specimens which I have examined are distinctly flattened, having a thickness less than one-half the width. In the size of sexually mature individuals there is a great variation. In the smallest worms examined the folds of the uterus were crowded with fully developed eggs. One of these specimens measured only 1.8 mm. in length and 0.67 mm. in width at the region of the testes. One of the largest mounts measured 5.8 mm. in length by 1.96 mm. in greatest width. Figure 3 shows an individual of about medium size. Measurements of a series of cross sections show the relation between width and thickness. A cross section thru the middle of the pre-acetabular region had a width of 0.94 mm. and a thickness of 0.48 mm.; at the acetabulum the width was 1.1 mm. and the thickness 0.48 mm.; at the anterior testis the width was 1.22 mm. and the thickness 0.54 mm.; at the posterior testis the width was 1.25 mm. and the thickness 0.54 mm.; and back of the posterior testis the width was 1.1 mm. and the thickness 0.44 mm.

The acetabulum in this species is located about one-third or one-fourth of the distance from the anterior to the posterior end. The ratio of the oral sucker to the acetabulum averages 4:3 and the

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oral sucker to the pharynx about 2:1. The ratio in specimens of different sizes varies but little altho the actual size of the suckers varies with the size of the animal.

The cuticula of *P. similiplexus* has a thickness of 0.007 to 0.012 mm. The region in front of the posterior testis is set with small spines which vary from 0.009 to 0.013 mm. in length (Fig. 4, *B*). These spines are more numerous around the anterior tip and become very scattered back of the acetabulum, and in the region back of the posterior testis the cuticula is entirely smooth. The above description differs considerably from Stafford's account (1902:907) of the cuticula and spines in this species. His description is as follows:

"The cuticule in preserved specimens is about 0.018 mm. thick and is regularly and thickly beset with spines, about 0.022 mm. in length, leaning backward with a slight curvature and extending thru the whole thickness of the cuticula, with the points projecting beyond. Viewed from the surface where one can see great numbers of them together, they appear to be in longitudinal and transverse rows, often 0.015 mm. to 0.02 mm. apart, but sometimes even less, or more, depending on the region and the state of contraction of the animal. As is common they are most abundant at the anterior end."

The genital field (Fig. 3) not including the vitellaria occupies about the third and fourth fifths of the body length. The ovary and testes are round or oval, small compact unlobed structures, which are definitely separated from each other. The small size, regular outline and position of these organs are characteristic of the species. The seminal receptacle is an oval structure ventrad of the ovary in the median line of the worm and overlapping for part if its length the posterior portion of the ovary. In a small sexually mature specimen 1.9 mm. long the ovary measured 0.29 mm. in length by 0.21 mm. in width, the anterior testis 0.34 mm. in length and 0.34 mm. in width and the posterior testis 0.46 mm. in length by 0.34 mm. in width. In a very large specimen 6.4 mm. long the ovary measured 0.46 mm. in length by 0.43 mm. in width, the anterior testis 0.48 mm. in length by 0.45 mm. in width, and the posterior testis 0.56 mm. in length and 0.58 mm. in width. The

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posterior testis was in every case larger than the anterior. While the ovary and testes were actually larger in the larger specimens than in the smaller, in proportion to the size of the animal they were always larger in the small individuals. In a section thru the ovary 1.1 mm. wide and 0.54 mm. thick the ovary was 0.26 mm. wide and 0.26 mm. thick. The testes fill almost the entire thickness of the posterior body region. In a cross section thru the posterior testis 1 mm. wide by 0.37 mm. thick the testis had a thickness of 0.33 mm.

The vitellaria (Fig. 3) in *P. similiplexus* are characterized by the large size of the acini and the small number in a group. In average sized specimens the acini have a length varying from 0.096 to 0.14 mm. and a width from 0.06 to 0.1 mm. The number in a group varies from 6 to 13 with the average number about 10. The groups (Fig. 3, *Y*) extend from about half way between the anterior end and the acetabulum to within a short distance of the posterior tip. There are always at least two groups behind the posterior testis between the intestinal ceca, and two or three median groups at the anterior end. In five specimens where the complete arrangement could be made out there was found to be considerable variation. The total number of groups varied from 16 to 19, with six or seven on a side beside the median groups. Stafford notes that the vitellaria in this species extend to the ends of the intestinal ceca. His drawing however shows them extending only to the region of the posterior testis. Klein (1905:64) at the end of his description of a new species of this genus, sums up the specific differences of all the species of the genus *Pneumonœces* in a table for comparison. As one characteristic of *P. similiplexus* in order to bring it into close relation to *P. similis* Looss he gives the following:

"Dotterstöcke reichen nicht bis zum hindern Rand des hindern Hodens;" but he adds this note:

"Stafford gibt in seiner Arbeit 1902 an, dass die Dotterstöcke bei *Pneumonœces similiplexus* bis ins Hinderende des Körpers, jedenfalls bis zum Ende der Darmschenkel reichen; diese Bemerkung stimmt jedoch nicht mit seiner eingnen Abbildung überein, da letzere gleiches Verhalten zeigt wie bei *Pneumonœces similis*."

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That Klein should have chosen this characteristic from Stafford's description rather than his drawing is shown by the extent of the vitellaria in the specimens of this species which I have examined.

The folding of the uterus is characteristic. From its beginning in the shell gland the uterus passes for a short distance forward, and then goes to the right side and loops down in a slightly dorsad position to the region back of the posterior testis where it fills the space between the intestinal ceca. From the posterior end of the body it forms two longitudinal folds outside the intestinal ceca, which reach beyond the anterior margin of the posterior testis. The ascending part of the uterus passes forward ventrad of the descending loops and between the testes to the right side of the body where it runs longitudinally for a short distance. Next it passes to the left side just behind the ovary dorsad of the acetabulum and fills with short transverse folds the region between the intestinal ceca up to the genital pore. The above description holds good for those individuals in which the ovary is on the right side of the body. The course of the uterus is exactly reversed when the ovary is to the left.

The eggs of this species are the largest of the American species of the genus (Fig. 1, *A*). Fully developed eggs vary from 0.034 to 0.04 mm. in length and from 0.017 to 0.21 mm. in width with an average of 0.0376 mm. in length and 0.0184 mm. in width.

Pneumonœces medioplexus Stafford 1905
Syn. *Hæmatolœchus medioplexus* Stafford 1902
Ostiolum formosum Pratt 1903

DIAGNOSIS. Characters of genus: long slender worms; cuticula covered with small spines; ratio of oral sucker to acetabulum greater than 4:1; ovary and testes unlobed; acini of vitellaria large with from 6 to 13 in a group; no longitudinal folds of uterus outside intestinal ceca; eggs average 0.032 mm. in length and 0.0186 mm. in width; in lungs of *Rana pipiens.*

DESCRIPTION. *P. medioplexus* was first reported from Canada by Stafford (1902:908) from the lungs of *Rana virescens* Kalm. One year later the same form was described by Pratt (1903:34) as *Ostiolum formosum.* This species has proven to be by far the

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most common fluke in the lungs of specimens of *Rana pipiens* which I have examined. I should expect to find it widely distributed throughout the range of *Rana pipiens*. Infection is sometimes very heavy, records showing as many as thirty or forty flukes in one frog. The worms are firmly attached by the oral sucker to the walls of the lung, and their bodies hang free in the cavities. They are very active either when attached or freed, showing great power of extension and contraction of the body, and a very free movement of the pre-acetabular region.

P. medioplexus is an elongate slender worm (Fig. 2) widest at the region of the anterior testis. One of the largest mounts had a length of 7.8 mm. and a width at the region of the anterior testis of 1.2 mm. From the ovary back to the limit of the posterior testis the width is about uniform, the pre-acetabular region is considerably attenuated and the region back of the posterior testis narrows to a bluntly pointed posterior tip. The smallest sexually mature specimen examined had a length of 3.9 mm. and a greatest width of 0.59 mm. Stafford's notes that living specimens of this species may reach a length of 15 or 16 mm.

This worm is distinctly flattened, the thickness being on the average about two-fifths of the width. Measurements of a series of cross sections of a single worm show this relation. In a section in the middle of the pre-acetabular region the width was 0.66 mm. and the thickness 0.33 mm.; at the acetabulum the width was 0.93 mm. and the thickness 0.38 mm.; at the middle of the ovary the width was 0.94 mm. and the thickness 0.42 mm.; thru the middle of the anterior testis the width was 1.1 mm. and the thickness 0.45 mm.; thru the posterior testis the width was 1.12 mm. and the thickness 0.46 mm.; and half way between the posterior testis and the posterior end of the body the width was 0.94 mm. and the thickness 0.38 mm.

The genital field without the vitellaria occupies a little more than the second third of the body length.

The acetabulum is separated by quite a little distance from the anterior margin of the ovary and is in front of the first third of the body length. It is very small being only about one-fourth or one-fifth the size of the oral sucker. In a specimen in which the

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oral sucker measured 0.39 mm. by 0.37 mm. the acetabulum had a diameter of 0.08 mm. The pharynx is about three-fourths the size of the oral sucker. In a specimen 7.8 mm. in length the oral sucker had a length of 0.40 mm. and a width of 0.35 mm. and the pharynx had a length of 0.29 mm. and a width of 0.26 mm.

The cuticula of this species measures on the average 0.007 mm. in thickness and is densely covered with a thick coating of spines about 0.01 mm. in length (Fig. 4, *C*). Stafford very aptly describes them as looking under low power "like a dense coat of short hair." They cover the entire surface of the body and thin out but little toward the posterior end. Pratt (1903:36) makes the absence of spines an important diagnostic character of *Ostiolum formosum* in fact using it as one of the distinguishing characters of his new genus. The examination of his type specimens showed that the cuticula was entirely sloughed off. This would account for the absence of spines in his description.

The testes (Fig. 2, *T*) are unlobed round or oval with somewhat squared corners and separated sometimes by a distance equal to half the length of one of them. In the large specimen previously mentioned, 7.8 mm. in length the anterior testis is 0.56 mm. long and 0.64 mm. wide and the posterior testis has a length of 0.64 mm. and a width of 0.6 mm. The thickness of the testes is but little less than their width and is practically equal to the thickness of the body. In a cross section thru the middle of the anterior testis 1.1 mm. wide by 0.41 mm. thick the testis had a width of 0.45 mm. and a thickness of 0.36 mm. The relations of the male ducts in this species show little of specific value. Figure 11 shows the ends of both the male and female ducts.

The ovary (Fig. 2, *O*) is elongate oval, lying a little distance back of the acetabulum either to one side or the other of the body, with its long axis diagonal to the long axis of the worm and its anterior end toward the midline of the body. The shape of the ovary varies considerably and in several cases it was slightly lobed. In the same specimen for which the measurements of the testes were given the ovary had a length of 0.56 mm. and a width of 0.28 mm. This organ has a thickness equal to its width. In a cross section through the middle of the ovary 0.96 mm. wide by 0.40 mm. thick the ovary

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had a width of 0.29 mm. and a thickness of 0.30 mm. In about half the specimens examined the ovary was to the right side, in the other half it was to the left. The seminal receptacle is larger than the ovary and lies in the midline of the body posterior and dorsad of the ovary and overlapping it for about half its length. The relations of the ducts of the female organs are shown in Figure 13 and their characters are of generic rather than specific value.

The acini of the vitellaria (Fig. 2, *Y*) in *P. medioplexus* are large, about the same size as those of *P. similiplexus*, and there are from 6 to 13 in a group. The groups extend from a point about half way between the anterior tip and ovary to the region back of the posterior testis. It was possible to work out in detail the arrangement of the vitelleria in twelve specimens. Altho considerable variation was found in the number of groups and their position, in all specimens the general grouping was the same. Most of the groups are located in lateral lines along the sides of the body. The most anterior of the groups in each line were always about on a level. The line on the same side as the ovary was the shorter, extending only to the posterior limit of the anterior testis. The longer line on the opposite side always extended beyond the posterior testis with usually a median group connected with the last two posterior groups to form a triangle (Fig. 2, *Y*). One or two median groups were always present in the pre-ovarian field. In the twelve specimens studied the total number of vitellarian groups varied from 18 to 23. The variations in arrangement are to be found in the number in each lateral line and in the number of the median groups. The following table gives the details of arrangement for the twelve specimens.

	1	2	3	4	5	6	7	8	9	10	11	12
Total number	18	20	21	21	22	21	23	21	21	19	18	20
Number on Long Side	8	9	8	10	11	10	13	10	10	10	9	11
Number on Short Side	8	8	10	8	8	8	8	9	8	7	7	8
Number of Anterior Median Groups	1	2	2	2	2	2	1	2	2	2	2	1
Number of Posterior Median Groups	1	1	1	1	1	1	1	0	1	0	0	0

There is some question in regard to the value of the characteristics of the vitellaria of the trematodes for specific diagnosis.

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Barker (1907) finds considerable variation in these structures among certain species of the genus *Opisthorchis*. I have insufficient material for any detailed analysis of the vitellaria for the genus *Pneumonœces*. In the case of *P. medioplexus* where the largest number of specimens could be studied in detail the variations of the vitellaria fall within narrow limits. The general size of the acini, their number in a group and the number of groups are items which, though somewhat variable, yield definite points of specific difference. It may be suggested that the characters of the vitellaria of a trematode must not be given too much weight in the differentiation of species unless the variations have been worked out in a number of individuals.

The arrangement of the folds of the uterus of this species is very characteristic (Fig. 2, *U*). The uterus at its beginning runs ventrad for a short distance and then courses backward in short transverse folds between the testes, and down one side of the post-testicular region and back the other, filling all the space inside of the intestinal ceca. From the posterior limit of the testes the ascending folds pass between these organs ventrad of the descending folds, then forward ventrad of the seminal receptacle and ovary and up to the genital pore, filling the pre-ovarian space in the larger animals with short transverse folds. The general course of the folds is transverse and at the posterior end there are no longitudinal folds outside of the intestinal ceca. In two very large specimens examined there was a variation in this respect, for on each side in these specimens very short longitudinal folds extended outside of the intestinal ceca. These specimens showed the greatest mass of eggs and the most complicated coils of any of the specimens of this species examined. This variation emphasizes the tendency of the uterus in its development to crowd into every available space. The smallest specimen of this species examined was not half the length of the largest individuals but had mature eggs in the uterus up to the genital pore. In this individual the general course of the uterus was like that described above, but the transverse folds were shorter and much less complex.

The measurements of over two hundred eggs from several different individuals give a variation in length from 0.022 to 0.029

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mm. and a range in width from 0.013 to 0.017 mm. The average length was found to be 0.0255 mm. and the average width 0.015 mm. (Fig. 1, *D*). Stafford gives the size of the eggs in this species as 0.028 mm. by 0.018 mm. while Pratt gives the measurements of the eggs for his *Ostiolum formosum* as 0.039 mm. by 0.017 mm. This measurement proved to be an error since in the type specimen of *O. formosum* which I examined the size of the eggs was found to fall within the limits for the species as given above. Pratt has since re-measured the eggs for *O. formosum* with the same results. He ascribes his original mistake to a confusion of notes.

The study of Pratt's type specimen of O. *formosum* establishes its identity with the species *Pneumonæces medioplexus*. Since Stafford has priority his specific name stands. There remains to be considered the further question of whether this species should be separated from the genus *Pneumonæces* and be made the basis of a new genus as Pratt has done. At the time of Pratt's description of this form he knew only of the three European species of the genus *Pneumonæces*. The increased knowledge of these forms in my opinion invalidates Pratt's genus *Ostiolum* and forces us to include the form which he would place in this genus in the genus *Pneumonæces*. Pratt separates the new genus *Ostiolum from Pneumonæces* on the basis of the following characteristics. "It [*Ostiolum formosum*] differs principally from the genus *Hæmatolœchus* [*Pneumonæces*] in the position of the acetabulum, which is further forward than in that genus, the size of the testes, which are much smaller than in *Hæmatolœchus,* in the arrangement of the uterine folds, which have a general longitudinal direction in *Hæmatolœchus,* and in the length of the excretory vesicle which extends much further forward than in *Hæmatolœchus*. In that genus also the worms are also often covered with spines, while in *Ostiolum* these structures did not appear in any of the specimens examined by me."

An analysis of the above characters shows that if they are to be made the basis of generic distinction, the genus *Pneumonæces* would need to be split into three or four different genera, which would be absurd in view of the fundamental resemblances. The

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position of the acetabulum varies slightly in the different species of this genus depending on the amount of attenuation of the anterior end. Variations in the size and the shape of the testes are more properly specific than generic differences. The arrangement of the coils of the uterus seems to depend on the size and shape of the organs and the shape of the body since it grows into all the available space between the reproductive organs. The difference in the length of the median stem and branches of the excretory vesicle is not as great as Pratt's emphasis on that point would suggest and seems to depend on the length and amount of attenuation of the body. In his description of the excretory vesicle of *Ostiolum formosum* Pratt (1903:35) states that the median portion is close to the ventral side of the animal and that the branches are dorsad of the intestinal ceca. The fact is that in all the species of the genus Pneumonœces the median stem of the excretory vesicle is near the dorsal body wall and the branches ventrad of the intestinal ceca, and in Pratt's type specimen mentioned several times already there was no variation from the usual condition. The final point of difference made by Pratt in regard to the absence of spines has been shown to be based on an error. But at any rate since in the different species of the genus *Pneumonœces* there is great variation in the character of the spines the distribution of these structures in this genus must be considered to be of specific rather than generic value.

Pneumonœces complexus Seeley 1906

DIAGNOSIS. Characters of the genus; cuticula entirely without spines; ratio of oral sucker to acetabulum about 4:3; ovary and testes irregular in shape and may be slightly lobed; acini of vitellaria large with 12 to 17 in a group and four to six median groups in front of acetabulum; average size of eggs 0.032 by 0.018 mm.; no longitudinal folds of uterus outside of intestinal ceca; habitat lungs of *Rana pipiens*.

DESCRIPTION. Seeley (1906:248) described *Pneumonœces complexus* from the mouth of *Rana pipiens* from North Carolina. Since that time I have obtained several specimens of this species from the lungs of *Rana pipiens* from Raleigh, North Carolina. These specimens were very much distorted and crowded with eggs

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so that the internal organs were obscured and so preserved that it proved impossible to section any of them. However enough can be made out to add several points to Seeley's description, correct some errors and to give this form a place as a distinct species of the genus *Pneumonæces*. I will be glad to obtain better material so that the description can be carried further.

The presence of the specimens found by Seeley in the mouth of the host was without doubt an abnormal circumstance due to the fact that the frogs had been killed with chloroform, giving the worms a chance to migrate from the lungs. The normal shape cannot be determined from the distorted specimens at my disposal. The largest of my specimens had a length of 5.2 mm. and a width of 2 mm. Seeley's specimens were not so contracted. He gives measurements of 5.8 mm. in length, 1.7 mm. in width and 0.71 mm. in thickness. In shape he records them as being widest just in front of the middle of the body, tapering slightly to a blunt posterior end and rapidly toward the anterior end. The oral sucker is about twice the size of the pharynx, and bears a ratio to the acetabulum of 4:3. In the specimen 5.2 mm. long the oral sucker had a length of 0.39 mm. and a width of 0.38 mm., the pharynx a length of 0.22 and a width of 0.21 mm., and the acetabulum a diameter of 0.31 mm. The cuticula in this species is entirely smooth. The ovary and testes are rather irregular in shape and slightly lobed. The testes overlap for part of their lengths.

The vitellaria have from 12 to 17 acini in a group and the acini themselves have a length in average sized individuals of 0.08 to 0.14 mm. and a width varying from 0.056 mm. to 0.096 mm. The groups reach from just a little way back of the pharynx to near the posterior end. They show a greater massing of groups in the anterior end than in any of the other species studied, there being 9 or 10 groups in front of the ovary, 4 to 6 of which are median. The total number of groups in two specimens in which they could be completely made out was 22 and 23.

The uterus in this species has no lateral longitudinal folds outside the intestinal ceca.

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The eggs of *P. complexus* (Fig. 1, *C*) have a length varying from 0.030 to 0.035 mm. and a width from 0.017 to 0.020 mm. The average length is 0.032 mm. and the average width is 0.0186 mm.

Strange to say, Seeley makes no special mention of three rather important characteristics of *P. complexus* as described by him. According to his description and figure (Seeley, 1906:248) this worm has no cirrus or cirrus sac, the genital pore to one side of the median line and the yolk glands on the ventral side of the body. Since Seeley seems to ascribe no particular value to the differences noted above, since he very evidently had insufficient and poor material and since his paper gives evidence of superficial observation and lack of grasp of the subject, I should be inclined to ascribe these differences to mistakes in observation. The specimens I have examined of this species are like the others of the genus in these regards.

Pneumonœces coloradensis n. sp.

DIAGNOSIS. Characters of genus; elongate slender worms; thickness greater than half width; spines present only on anterior tip; ratio of oral sucker to acetabulum 5:4; field of genital glands, without vitellaria, less than one-third body length; ovary and testes round to oval to slightly irregular, but unlobed; testicular zones usually abut; groups of vitellaria in front of acetabulum few; no longitudinal folds of uterus outside intestinal ceca; eggs average 0.0344 mm. in length and 0.0195 mm. in width; habitat lungs of *Rana pipiens*.

DESCRIPTION. *Pneumonœces coloradensis* n. sp. was first brought to my attention by a mounted specimen given me in September 1913 by Dr. M. C. Hall from material collected from Boulder, Colorado. Later the same fall I came upon this species again in the lungs of specimens of *Rana pipiens* from a small ditch east of Colorado Springs, Colorado. At this time in all forty-seven specimens were obtained from nine out of seventeen hosts examined. In the summer of 1914 further examination was made of the frogs from the region of Colorado Springs, showing that in *Rana pipiens* this new species was comparatively common. In fact it is the only frog lung fluke found in this region. In one of the frogs examined in the late summer of 1914 fifteen flukes of this species were found,

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showing gradations in size from a little beyond the cercarial stage to very large adults. As in other species of the genus there is considerable variation in the size of sexually mature individuals, the largest being as much as twice the length of the smallest. The largest mount examined measured 8.1 mm. in length and 1.55 mm. in width, while the specimen shown in figure 8 with a fully developed uterus and mature eggs up to the genital pore, measured 3.3 mm. in length and 0.54 mm. in width. In shape this species is most like *P. mediolexus,* being narrow for its length but tapering less toward the ends. The region from the anterior groups of the vitellaria almost to the posterior end has a nearly uniform width. The thickness of the body is greater than half the width in every region except back of the posterior testis. A series of sections of a medium sized worm gave the following measurements: a section through the acetabulum was 0.7 mm. wide and 0.49 mm. thick; one through the region half way between the oral sucker and the acetabulum was 0.56 mm. wide and 0.48 mm. thick; through the middle of the anterior testis the width was 0.77 mm. and the thickness 0.48 mm.; through the middle of the posterior testis the width was 0.8 mm. and the thickness 0.5 mm.; and a section through the region half way between the posterior testis and the posterior end had a width of 0.64 mm. and a thickness of 0.3 mm.

The average ratio of the oral sucker to the pharynx is 10:7 and to the acetabulum 5:4. The ratio in both cases is quite constant. The ratio of the oral sucker to the pharynx varies from 8:5 to 5:4 and that of the oral sucker to the acetabulum from 7:6 to 4:3. The size of the suckers and the pharynx in sexually mature worms varies directly with the size of the worms, and there was no relation between the variations in size and of ratio of these structures.

The cuticula in *P. coloradensis* is smooth except at the very anterior tip in the region over the oral sucker and the pharynx. The spines (Fig. 4, *D*) covering this region are thickly set over the oral sucker and thin out rapidly backward, only a few scattered spines showing back of the pharynx. These spines are the largest for any of the species studied, averaging between 0.009 and 0.013

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mm. in length and from 0.003 to 0.005 mm. in diameter at their bases. They point strongly backward and have their points curved backward.

The ovary (Fig. 8, *O*) of this species is just behind the acetabulum which if further back than in any of the other forms studied, being just behind the limit of the first third of the body length. The field of the genital organs, without the vitellaria, is characterized in being further back and shorter than in any of the other American representatives of this genus. The distance from the anterior margin of the ovary to the posterior margin of the posterior testis is less than one-third of the total body length.

The ovary is a round or oval unlobed organ which lies just behind the acetabulum to one side or the other of the body. Ventrad of the ovary but somewhat posteriad and overlapping for about one-half its length is the seminal receptacle, which is a round or oval organ lying in the midline of the body, and a little larger than the ovary. The testes (Fig. 8, *T*) are round or oval to almost rectangular structures—close together with the two testicular zones usually abutting. They occupy approximately the fourth fifth of the body length and are only a little to the side of the median line. This position as in *P. **medioplexus*** depends on the narrowness of the body in this region. The posterior testis is the larger of the two. The size of the reproductive glands varies directly with the size of the individuals and will be given for two different specimens. In the mount 8.1 mm. in length the ovary had a length of 0.49 mm. and a width of 0.42 mm., the anterior testis was 0.64 mm. in length and 0.60 mm. in width and the posterior testis 0.74 mm. in length and 0.86 mm. in width. In a much smaller specimen 4.4 mm. in length the ovary had a length of 0.32 mm. and a width of 0.22 mm., the seminal receptacle had a length of 0.32 mm. and a width of 0.24 mm., the anterior testis had a length of 0.42 mm. and a width of 0.46 mm., and the posterior testis had a length of 0.46 mm. and a width of 0.44 mm. The testes have a thickness but little less than their width and almost equal to the thickness of the body at the testicular region. In a cross section thru the middle of the posterior testis having a width of 0.9 mm. and a thickness of 0.48 mm. the testis was 0.55 mm. in width and 0.46 mm. in thickness.

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The arrangement of the ducts of the reproductive organs is similar to that of *P. medioplexus* and offers no points of importance for specific diagnosis.

The vitellaria are almost entirely limited to the lateral zones, there being at most only one median anterior group and one or two posterior median groups which are always considerably to one side of the median line. The acini have about the same average size as those of *P. medioplexus* and there are from 8 to 14 in a group.

The coiling of the uterus is similar to that of the other narrow forms, *P. medioplexus* and *P. complexus*, there being no longitudinal folds outside of the intestinal ceca and the direction of the folds being transverse.

The eggs vary in length from 0.032 to 0.039 mm. and in width from 0.018 to 0.021 mm., with an average length of 0.0344 mm. and an average width of 0.0195 mm.

A considerable series of immature forms of this species was taken from the lungs of the frogs. The youngest specimen found (Fig. 5) which is by far the youngest specimen of the genus ever reported, was found with a number of other specimens of the same species in the lung of a specimen of *Rana pipiens*. There is no question in my mind that this specimen belongs to the species *Pneumonœces coloradensis* since it was found with immature and mature individuals of this species, and no other frog lung fluke has been found near Colorado Springs where this was collected. Since this immature form gives some hint of the character of the cercaria of this species an extended description of its structure will be given.

This specimen (Fig. 5) has a length of 0.4 mm. and a width of 0.15 mm. The acetabulum is back of the center of the body and has a diameter of 0.063 mm. The mouth opening is slightly subterminal. The oral sucker has a length of 0.079 mm. and a width of 0.074 mm. and the pharynx has a length of 0.0496 mm. and a width of 0.039 mm. It is interesting to note that the ratios of the oral sucker to the acetabulum and pharynx fall within the limits of the ratios found for these structures in the adults. The esophagus is very short and in the figure entirely contracted behind the pharynx. The intestinal ceca run almost to the end of the body of the animal and contain blood, showing that the worm had been in

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the frog's lung long enough to be feeding on the blood. The excretory pore is at the middle of the posterior tip. It connects with the main part of the excretory bladder by a short narrow tube, 0.016 mm. in length. The bladder consists of the wide median part and two wide lateral divisions extending up to the region of the oral sucker dorsad of the intestinal ceca. In the living animal all parts of this bladder were crowded with highly refractive concretions like those which gather in the excretory systems of cercariæ. This is the only immature stage in which I found those concretions. The excretory bladder resembled very closely the condition found in encysted Xiphidiocercariæ and this character together with the structure of the digestive system suggests that the cercariæ of the species of the genus *Pneumonœces* belong to this group. The locomotion of this immature specimen was watched on a substratum. With the acetabulum firmly attached the anterior end would stretch forward and the oral sucker would take hold. The acetabulum was then loosened and the body contracted. The acetabulum then took hold and the movement was repeated. By a rapid series of these movements this larval form could make considerable progress. This movement is exactly like that of a cercaria on a substratum. Dorsad of the acetabulum are irregular masses of nuclei which are probably the anlage of the reproductive organs. In the drawing (Fig. 5) they are hidden by the acetabulum.

Several larger immature specimens of this species were available for study. The form shown in figure 6 shows considerable increase in size over that shown in figure 5. The intestinal ceca are much distended with frog's blood hiding the excretory vesicle. The anlage of the reproductive system shows definite differentiation into organs. In a larger form (Fig. 7) which appears about ready to produce eggs, but having none in the uterus, the organs are fully developed and differ only in size from those of the sexually mature specimens. The folds of the uterus in this specimen are much less complicated than in those individuals where a quantity of eggs is present.

P. coloradensis is most closely related to *P. complexus*. It has the same shape of the body and general arrangement of the uterus as that species. The size of the eggs and the general relations of

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the reproductive organs are more like that species than any other. Our slight knowledge of *P. complexus* makes comparison of the two species in certain characters impossible. Enough is known however to make sure their distinctness. Differences between these two species are noted in the ratios of the oral sucker to the acetabulum and pharynx, and in the size of the eggs. *P. complexus* is entirely without spines, while *P. coloradensis* has spines at the anterior tip. The most noticeable difference is in the arrangement of the vitellaria. In *P. complexus* there are eight to ten groups of vitellaria in front of the acetabulum of which four to six are median groups, while in *P. coloradensis* there are not more than five groups in front of the acetabulum and in none of the specimens examined was there more than one median group.

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KEY TO THE AMERICAN SPECIES OF PNEUMONŒCES

1(2) Longitudinal folds of the uterus present outside the intestinal ceca reaching at least in front of the posterior testis..... 3

2(1) No longitudinal folds of the uterus present outside the intestinal ceca 7

3(4) Testes elongate, length at least twice width..... 5

4(3) Testes round or oval, length but little greater than width.....
Pneumonœces similiplexus Staf.

5(6) Longitudinal folds of the uterus outside the intestinal ceca reaching to the pharynx.
Pneumonœces longipplexus Staf.

6(5) Longitudinal folds of the uterus outside the intestinal ceca never reaching in front of ovary.
Pneumonœces breviplexus Staf.

7(8) Acetabulum very small, not one-fourth size of oral sucker.
Pneumonœces medioplexus Staf.

8(7) Acetabulum three-fourths size of oral sucker..... 9

9(10) Not more than two median groups of the vitellaria in front of the acetabulum.
Pneumonœces coloradensis Cort.

10(9) Four to six median groups of the vitellaria in front of the acetabulum.
Pneumonœces complexus Seeley

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Macalester College, Saint Paul, Minn.

ABBREVIATIONS USED IN PLATES

A, acetabulum
C, cirrus
CS, cirrus sac
DS, duct of seminal receptacle
E, excretory vesicle
G, genital pore
I, intestine
M, metraterm
MG, Mehlis' gland
O, ovary
OS, oral sucker
OT, oötype
P, pharynx
PG, prostate glands
S, seminal vesicle
SR, seminal receptacle
T, testis
U, uterus
VD, vitelline duct
VR, vitelline reservoir
Y, vitellaria

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EXPLANATION OF PLATES

All drawings were made with a camera lucida. For the sake of clearness the eggs are omitted from the uteri in the toto drawings.

Fig. 1. Eggs of the American species of Pneumonœces, average size.
A. Egg of *Pneumonœces similiplexus.*
B. " " " *coloradensis.*
C. " " " *complexus.*
D. " " " *medioplexus.*
E. " " " *longiplexus.*
F. " " " *breviplexus.*

Fig. 2. *Pneumonœces medioplexus,* dorsal view.

Fig. 3. " *similiplexus,* " "

Fig. 4. Cuticular spines of American species of *Pneumonœces.*
A. Spines of *Pneumonœces longiplexus.*
B. " " " *similiplexus.*
C. " " " *medioplexus.*
D. " " " *coloradensis.*

The cuticula of *Pneumonœces complexus* and *P. breviplexus* is without spines.

Figs. 5, 6, 7. Stages in the development of *Pneumonœces coloradensis.*

Fig. 8. Fully developed *Pneumonœces coloradensis,* ventral view.

Fig. 9. *Pneumonœces breviplexus,* dorsal view.

Fig. 10. " *longiplexus,* " "

Fig. 11. End passages of the reproductive system of *Pneumonœces medioplexus.*

Fig. 12. End passages of the reproductive system of *Pneumonœces longiplexus.*

Fig. 13. Diagramatic reconstruction of the connections of the female reproductive organs of *Pneumonœces medioplexus.*

Fig. 14. Diagramatic reconstruction of the connections of the female reproductive organs of *Pneumonœces longiplexus.*

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PLATE VII

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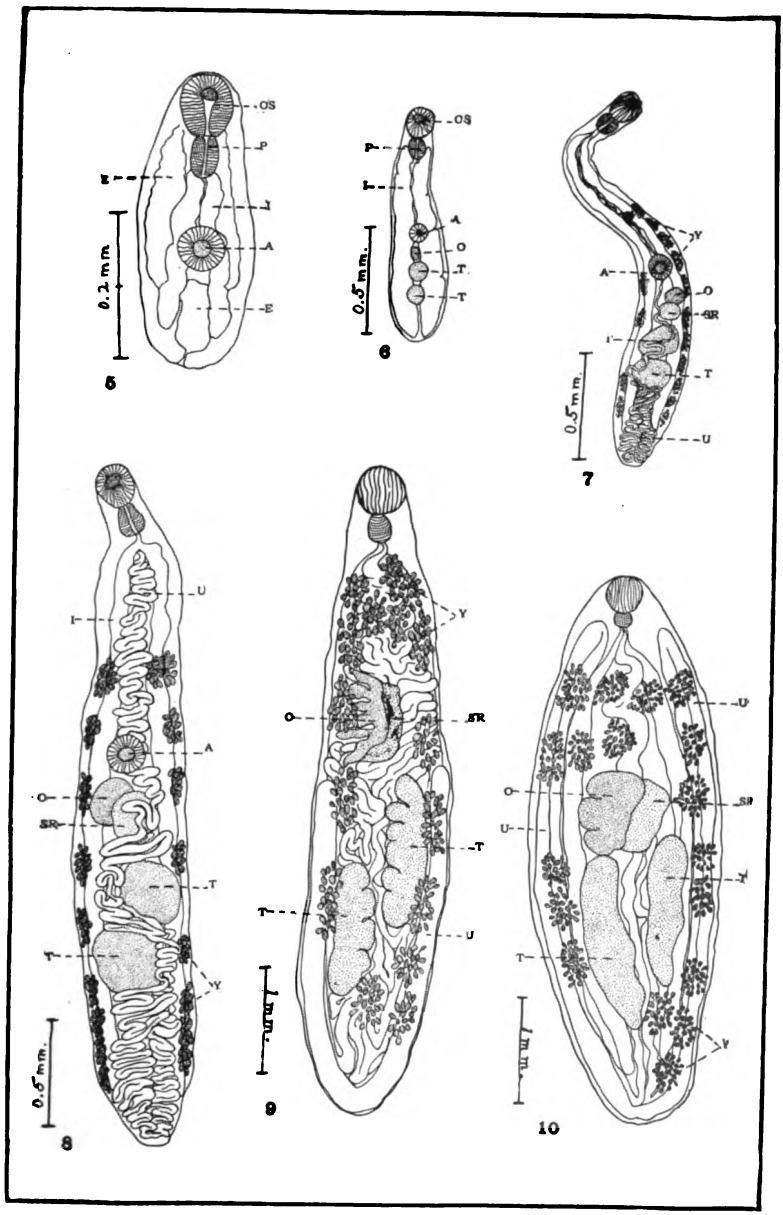

PLATE VIII

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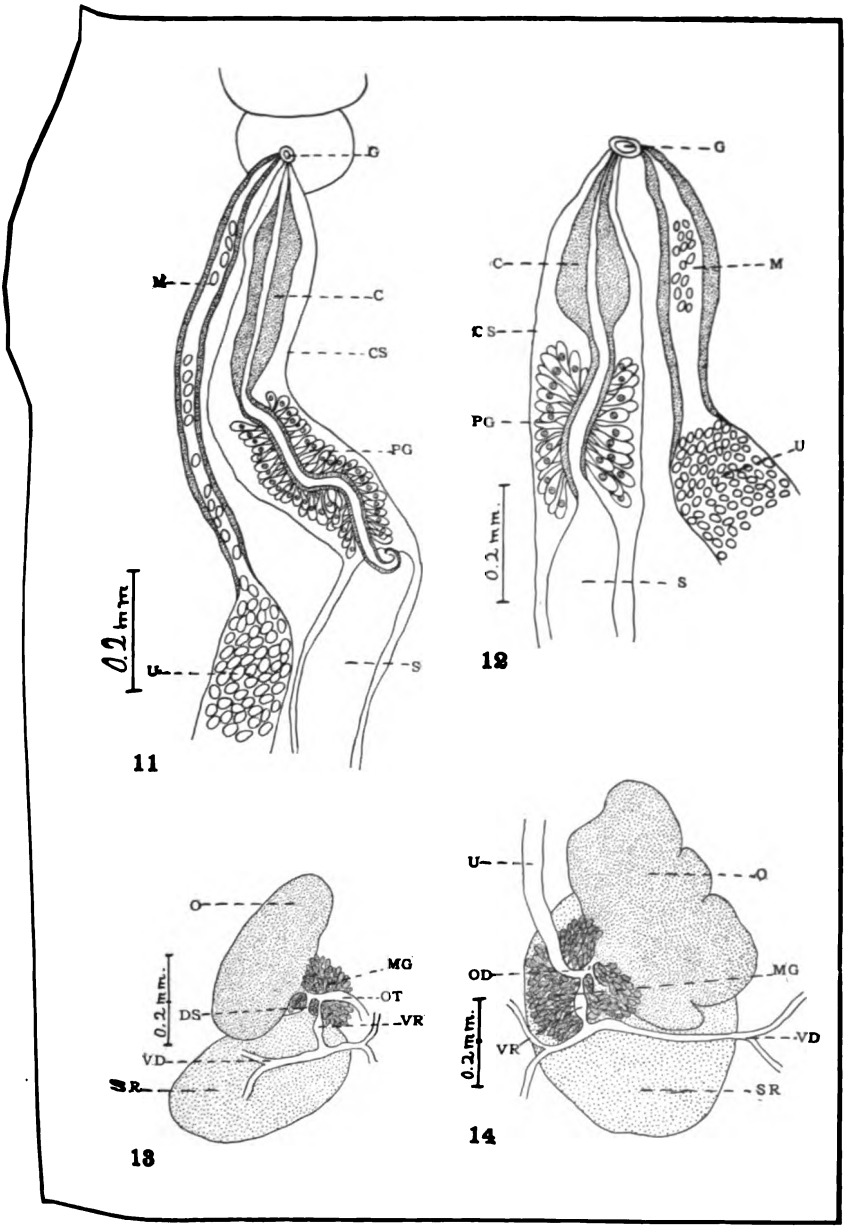

PLATE IX

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ELYTRAL TRACHEATION OF THE TIGER BEETLES (CICINDELIDÆ)*

By V. E. Shelford

I. Introduction

In course of a study of the relation of the various structures to the color pattern of Cicindela a large number of elytra were mounted in hot balsam and the trachea examined as was described in an earlier paper (Shelford '13). The studies embraced all of the families and subfamilies of the Cicindelidæ of earlier authors. The group is world wide and some of the material has been obtained only with expense and difficulty. The studies have involved the drawings of elytra of many individuals and the results as a whole show the tracheation of the elytra of a large heterogeneous group. A large number of drawings is presented because they are much more satisfactory than description, and some idea of variation and the form of the elytron can be obtained. Fully three times as many individuals as shown, were drawn outside of Cicindela.

The nomenclature used is from Horn's "Index der Cicindeliden". Elytra of Cicindela and Tetracha have been studied fresh, and the amount of asymmetry and variation of different individuals noted.

II. General Structure of the Elytron

1. *Chitinous Columns*

The two walls of the sac like elytron are held together by chitinous pillars or columns which in the adult appear in cleared elytra as shown in figure 1. The different layers of cuticula show here as rings around the original central spindle. These are often proportionately larger and occupy more of the space than shown in this figure.

*Contributions from the Zoological Laboratory of the University of Illinois, No. 54.

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2. *Hairs*

Hairs which in a primitive insect usually cover the wing entirely are present in nearly all tiger beetle elytra. In the Manticholas the hairs are of two sizes and are usually just in front of the chitinous columns. Hairs in this group are usually located on the posterior side of backward projecting tubercles. Under the microscope the hairs may be located on the pigmented area of the elytra by the light area which is produced by the thin cuticula at the base of each hair. Hairs appear on the whole to be less common in the unpigmented areas and when present usually are surrounded by a narrow rim of pigmented cuticula. Hairs occur in practically all groups though they have been lost from the majority except for a few at the base of the elytron and scattered along the trachea. (See fig. *6, 21-31, 32-42, and 45.*) In *Platychila pallida* I found no indication of hairs. This is the only representative of the Platychilidæ. *Mantichora* (*Mantichoridæ*) (Fig. 2) which stands in close relation to *Platychila* has the dorsal portion of the fused elytra covered very fully with hairs and the epipleuron has only scattered hairs. One of the *Megacephalidæ, Megacephala (Tetracha) æquinoctialis* has the elytron completely and uniformal covered with small hairs. The other species of Megacephala which I have examined *klugi* and *limata* show only a few hairs at the base of the elytron (Fig. *43* to *45*).

In the only observed representative of the *Pognostomidæ* which stand at the beginning of the series in W. Horn's classification,

---

FIGS. 1-7. STRUCTURE OF THE ELYTRON AND THE MORE GENERALIZED TYPES OF TRACHEATION

Fig. 1. Mantichora tuberculata DeGeer (Africa); Arrangement of columns and hairs.

Fig. 2. Mantichora tuberculata DeGeer (Africa); Distribution of hairs.

Fig. 3. Pognostoma chalybæum Klg. (Madagascar); Distribution of hairs.

Fig. 4. Amblychila cylindriformis Say (Kansas); Six fully developed trachea.

Fig. 5. Omus Dejeani Rehe (California); Degenerate radius and media.

Fig. 6. Collyris sp; Showing reduced cubitus and anal.

Fig. 7. Platychila pallida F. (S. Africa); Reduced anal.

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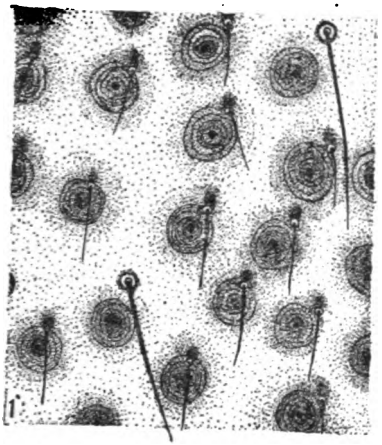
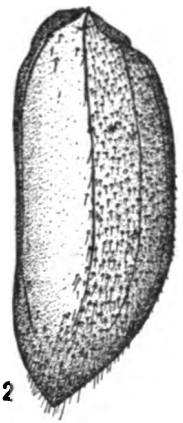
PALAEOMANTICHORIDAE

CTENOSTOMIDAE



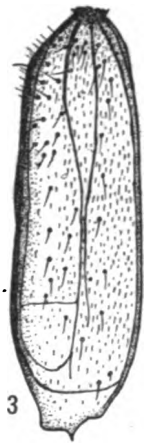
1

MANTICHORA



2

MANTICHORA



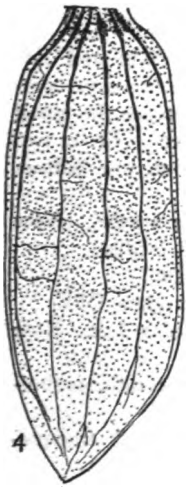
3

POGNOSTOMA

NEOMANTICHORIDAE

COLLYRIDAE

PLATYCHILIDAE



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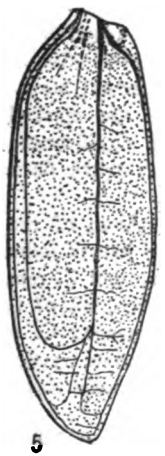
AMBLYCHILA



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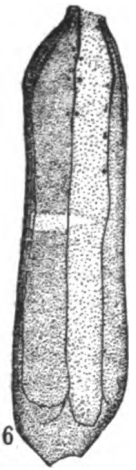

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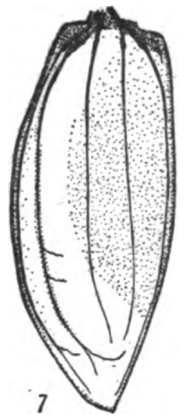
COLLYRIS



7

PLATYCHILA

PLATE X

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the elytra was covered with hairs. In the *Collyridæ (Collyris* and *Tricondyla* are arboreal) the hairs are confined to a few at the base and a few scattered along the lines of the tracheæ.

3. Pits and depressions

The elytra of many species are marked with pits. Close examination under the microscope with both transmitted and reflected light shows that, in the majority of cases, the pits are over the center of the chitinous columns and bear no relation to rudimentary hairs as Dr. W. Horn has suggested. I have seen no pits that would appear to represent rudimentary hairs though they may occur.

4. Veins and thickenings

There are sometimes thickenings running lengthwise of the elytron as in Domica (fig. 35 and 36). While these thickenings run parallel with the trachea they are usually between rather than coincedent with them, except in Caledonica (fig. 25). There are

---

FIGS. 8-19. ELYTRA OF FRESHLY EMERGED CICINDELAS

Fig. 8. Showing the media, cubitus, and anal (rudimentary in freshly emerged specimens of C. repanda. Dej.

Fig. 9. The same in a freshly emerged C. punctulata Oliv.

Figs. 10-11. Showing in full detail the tracheation of the right and left elytra of a freshly emerged C. Leconti Hald. Note the close similarity of the main trunks and the marked difference in small branches, indicating the lack of significance of these. From two camera lucida drawings of fresh elytra flattened out, one bottom side up.

Fig. 12. Camera drawing of another specimen of the same species. Note the fewer cross branches and the longer radius and media.

Fig. 13. Camera drawing of a freshly emerged C. repanda Dej.

Figs. 14-15. Camera drawings of two individuals of C. punctulata Oliv.

Fig. 16. Camera drawing of a freshly emerged C. cuprascens Lec. Note the degenerate media and heavy cross trachea.

Fig. 17. Camera drawing of a fresh C. hirticollis Say. Note condition similar to that in 16.

Figs. 18-19. Camera drawings of two different individuals of C. tranquebarica Herbst.

In these figures note the branching in every case near the curve of the posterior side of the elytron, and the subcosta ending at the same point with branches extending inward in every case.

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CICINDELIDAE CICINDELINI

CICINDELA

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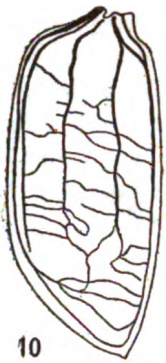

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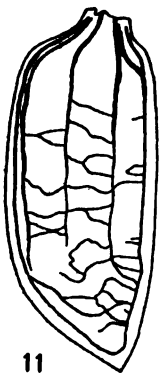

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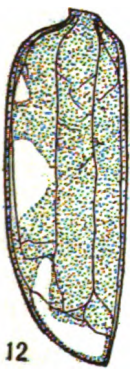

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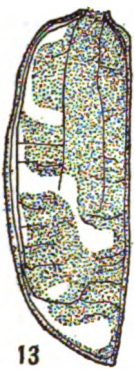

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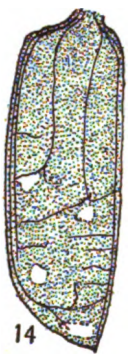

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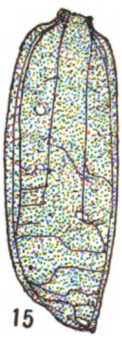

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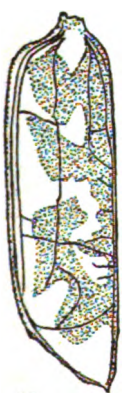

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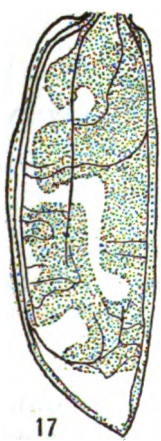

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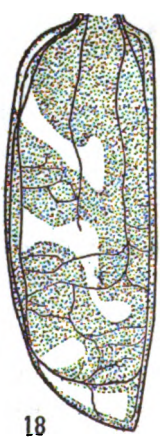

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19

PLATE XI

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however some thickenings on the under side of the elytra of most species which correspond in a general way to veins (particularly in Mantichora). The outer and inner margins of the elytra are always thickened and resemble veins almost invariably containing tracheæ. The subcosta usually follows the costa very closely at the base of the elytron but just behind the middle it turns inward away from the margin in a vein like thickening. The radius is in a distinct thickening of the elytron which proceeds from the base for a short distance. This is very constantly present. Aside from this nothing comparable to veins is present but the rows of chitinous columns are often so arranged so as to give distinct and direct spaces running the length of wing. These are occupied by the principal trachea. In some cases the spaces appear very clearly on the under side of the elytron and in Mantichora there are distinct ridges over them which have every appearance of veins.

III. Tracheation

1. The main trunks

The six trunks common in insects are represented in but two genera (*Amblychila* and *Mantichora*), (Figs. 4 and 6), which have rudimentary wings and specialized elytra fastened together in the

FIGURES 20-29. THERATIDÆ AND EURYODINI

Fig. 20. Therates basalis Dej. (New Guinea).
Fig. 21. Therates labiatus Fab. (Oriental region).
Fig. 22. Therates fasacitus Fabr.
Fig. 23. Eurosopus Chaudoiri, Thom. (South America).
Fig. 24. Eucallia Boussingaulti Cuer. (South America).
Fig. 25. Caledonica acentra Chd. with thickenings in the lines of the trachea. (New Caledonia).
Fig. 26. Distypsidera sp. (New Holland).
Fig. 27. Distypsidera undulata Westw. (New Holland).
Fig. 28. Prothyma adonis sb. sp. bellula Flt. (Madagascar).
Fig. 29. Prothyma versicolor Dej. (N. Africa).

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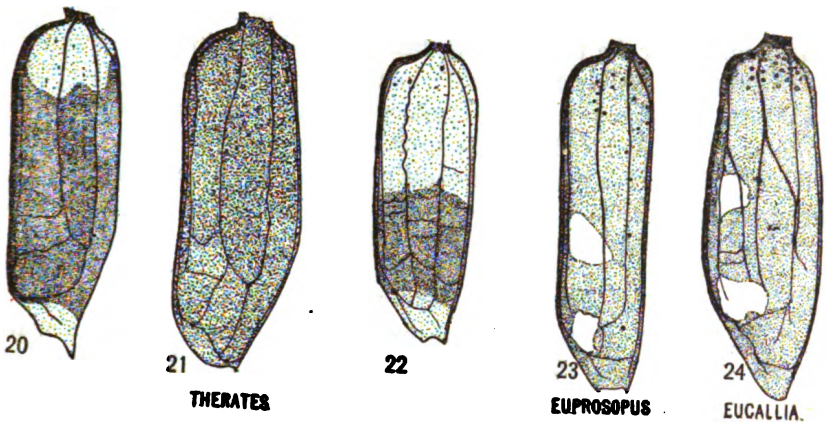
THERATIDAE

EURYODINI



20

21

THERATES

22

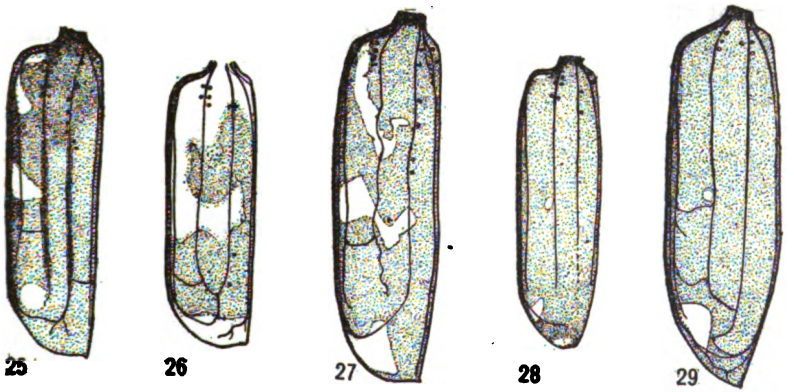
23

EUPROSOPUS

24

EUCALLIA.

EURYODINI



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CALEDONICA

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27

DISTYPSIDERA

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PROTHYMA

PLATE XII

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adult. These trachea are demonstrated in the adult dried elytra without any difficulty. In Omus which is closely related to Amblychila the radius and media have disappeared except for rudiments. The cubitus is the principal trachea (Fig. 5). With the exception of Omus and Amblychila it is the anal that has degenerated farthest. Collyris was never very satisfactory for study but it appears that the cubitus is reduced and the anal wanting (Fig. 6). In figure 7 *(Platychila pallida)* the commonest type of tracheation of the family and probably among the most generalized so far as the first four trachea are concerned, is shown. The anal is much reduced.

2. *Details of Tracheation in Cicindela*

Figures 8 and 9 show two different arrangements of the anal rudiment in Cicindela. In figure 8 it is joined with the cubitus. In figure 9 it is likewise joined in the same place but a small branch suggests a backward extension. The number of small branches and cross connections is large and too variable to be correlated with other specific characters. Figures 10-19 illustrate this fact. Figures 10 and 11 are the two elytra of an individual and show a marked difference. It is evident then that only the main trunks are at all constant. The costal branch at the center the posterior third of the elytron at the beginning of the curve is very characteristic of Cicindela.

FIGURES 30-33. ODOMTOCHILINI

Fig. 30. Oxygonia moronensis Bat. (South America).
Fig. 31. Odontochila punctum Klg.? (South America).
Fig. 32. Odontochila brasiliensis Dej. (South America).
Fig. 33. Prepusa ventralis Dej. (South America).

FIGURES 34-37. DROMICINI

Fig. 34. Dromica Kolbei W. Horn. (South Africa).
Fig. 35. Dromica sculpturata Boh. Thickenings shown as dark areas, not related to the trachea. (South Africa).
Fig. 36. Dromica tuberculata Dej. (South Africa).
Fig. 37. Dromica limbata Bert. (South Africa).

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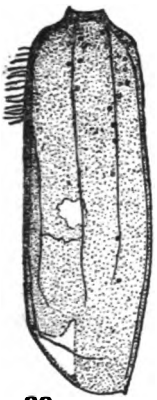
CICINDELIDAE

ODONTOCHILINI



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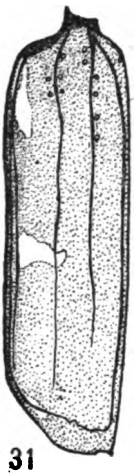
OXYGONIA



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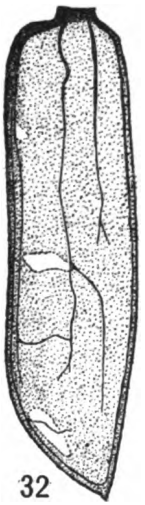
ODONTOCHILA



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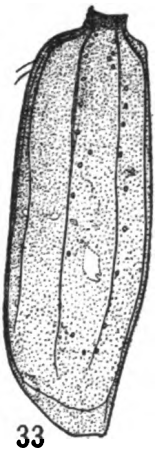
ODONTOCHILA



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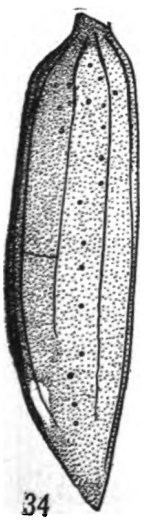
PREPUSA

CICINDELIDAE

DROMICINI

DROMICA



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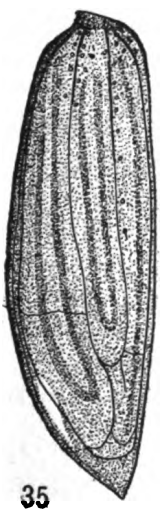

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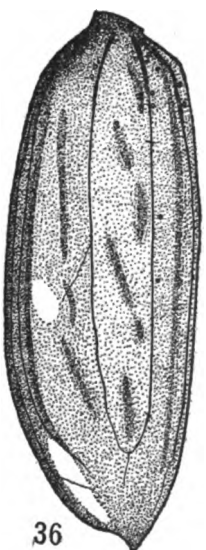

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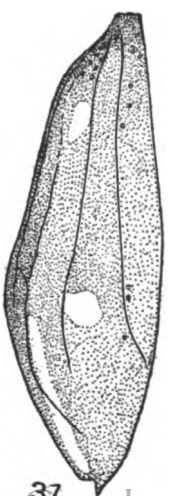

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PLATE XIII

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3. *Peculiarities of the different Taxonomic Groups*

The peculiarities or distinguishing features of Cicindela and of the Palæomantichoridæ, Neomantichoridæ, Pognostomidæ, Platychilidæ and Collyridæ have been noted. The remaining families or subfamilies still require mention.

a. Theratidæ. (Figs. 20-21) The radius and media unite in the center of the posterior third of the elytron and are united with the costa and subcosta by three or four branches.

b. Cicindelidæ-Euryodini. (Figs. 23-29) The main trunks are similar to those of Cicindela but differ characteristically in the absence of a costal branch or of its presence near the tip of the elytron than in any other subfamilies. Compare figs. 26-27 with 10-19. In fig. 24 are shown two backward branchings (such as occur in Cicindela). The media appears well developed and joined to the subcosta as a rule.

c. Odontochilini. These differ only slightly from the Euryodini but as a rule the media is reduced and the costa joined to the cubitus.

d. The Dromicini appear to have an unbranched and sometimes reduced costa.

e. The Megacephalidæ (Figs. 38-45) are characterized by a well developed media which is as a rule the most prominent trachea of the elytron. The costal branches are somewhat farther posterior than in Cicindela.

FIGURES 38-45. MEGACEPALIDÆ

Fig. 38. Pseudoxychila bipustulata Latr. (South America).
Fig. 39. Oxychila tristis Fabr. (South America).
Fig. 40. Tetracha carolina (Georgia).
Fig. 41. Tetracha carolina Linn. (N. Carolina).
Fig. 42. Styphloderma asperta Wat. (Africa).
Fig. 43. Megacephala (Phæoxantha) limata Perty (South America).
Fig. 44. Megacephala (Phæoxantha) æquinotialis Dej. (South America).
Fig. 45. Megacephala (Phæoxantha) Klugi Chd. (South America).

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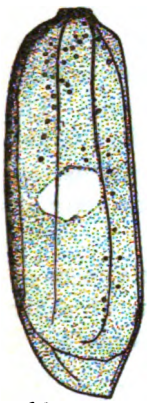
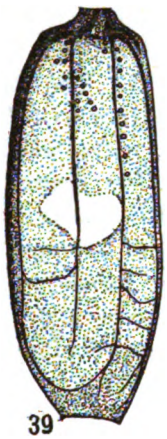


38

PSEUDOXYCHILA



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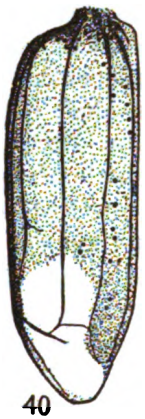
OXYCHILA



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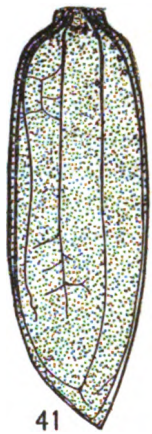
Tetracha Hope

MEGACEPHALA



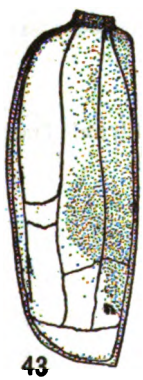
41

MEGACEPHALA

42

Stypholeorma Wat.

MEGACEPHALA



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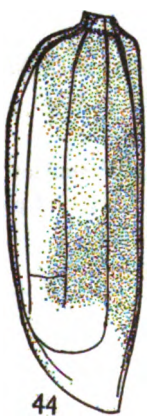
MEGACEPHALA



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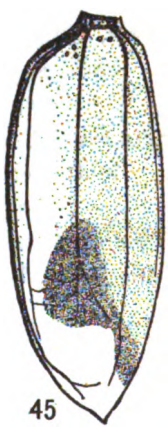
MEGACEPHALA



45

MEGACEPHALA

PLATE XIV

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In the set of figures facing this page the obvious and presumably more characteristic features of elytral tracheation, brought out in the preceding camera drawings are shown. The figures show characteristic differences between the different groups correlated with the general taxonomic differences. The notable characteristics of the family as a whole is the tendency to reduce the anal. The exception falls in the Mantichorid groups which are wingless and have the most specialized elytra. The figures show conclusively that tracheation is far from haphazard, that it is of a fairly constant taxonomic significance in this group of Coleoptera.

It has been shown that veins are formed in the development of elytra before the trachea enter. Accordingly no claim of exact homology with the corresponding veins of other orders is made.

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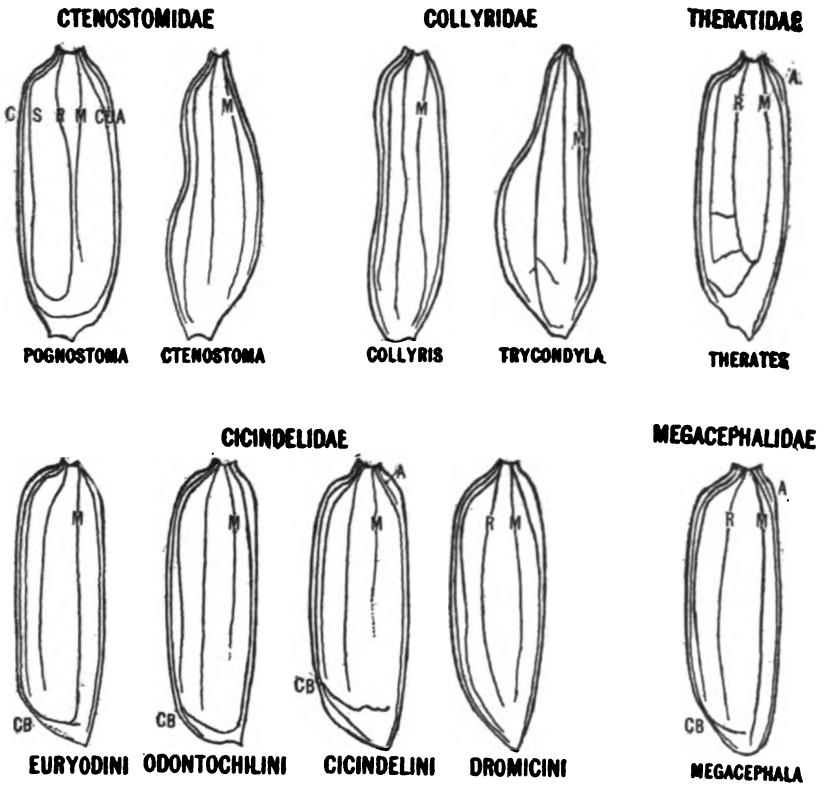

PLATE XV

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A STUDY OF THE SCALES OF SOME OF THE FISHES OF THE DOUGLAS LAKE REGION*

By Arthur T. Evans

During the summer of 1913 while in attendance at the University of Michigan Biological Station on Douglas Lake in Cheboygan County, Michigan, an effort was made to find out if possible whether the variation in the scales of different fishes is such that the scale characters are of any taxonomic value. The objects of the study were to determine, (1) whether there is a marked difference in the scales from different individuals of the same species; (2) whether the scales of an adult and a younger specimen of the same species varied sufficiently to be mistaken for scales of another species; and (3) to determine in just what way the scales of various families differ. As the length of time for conducting the work was limited only ten of the more abundant species were studied in detail.

The writer is indebted to Professor Max M. Ellis for help and suggestions in the course of the work.

Since the examination of all the scales of a number of fishes would be a big task and quite likely unnecessary it was decided to examine scales from what appeared to be the three most typical regions of the body of the fish. Before deciding upon these three typical regions an examination was made of the scales from various other parts of the body of the fish to determine just where the most variation occurred and of what this variation consisted. Throughout the course of the work whenever a fish of a different species was studied a complete examination was made of scales selected from various parts of the body to make sure that variation did not occur in parts not included in the typical bands. The three regions selected were, (1) just anterior to the dorsal fin (Band 1); (2) just anterior to the soft dorsal (Band 2); and (3) just anterior to the caudal fin. In each of these regions a band of scales was taken the entire distance around the body of the fish. Each scale was

*Contributed from the University of Michigan Biological Station Number 16.

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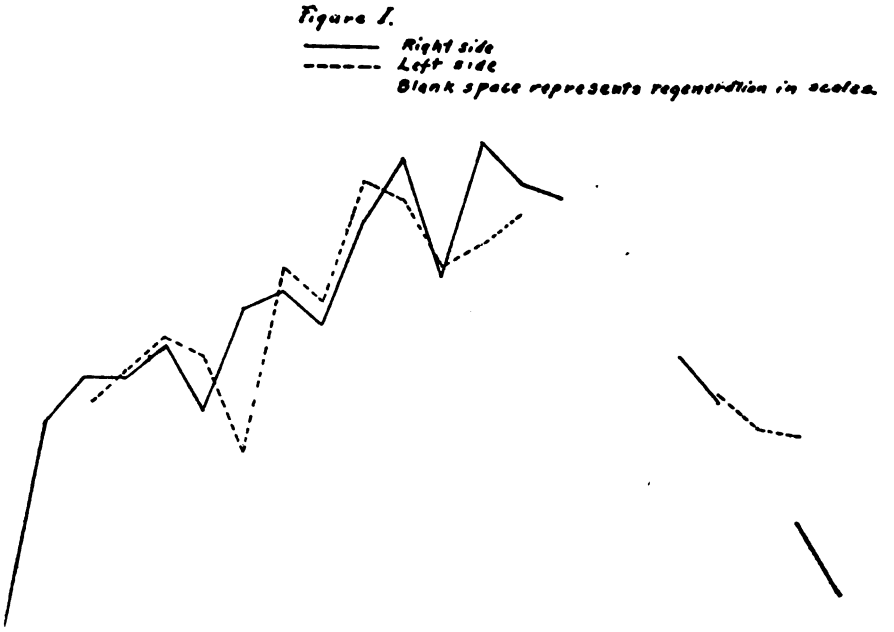
examined under the low power of the compound microscope and the following data recorded: (1) number of teeth, if any; (2) number of radii (this includes both entire radii and radii of which only parts were visible in Table 1); (3) number of basal lobes, (4) whether the circuli extended through the apical area or not, and (5) the form of the circuli.

It was supposed that there might be a variation in the number of teeth, radii, and lobes correlated with the age of the individual so in order to find out the exact extent of such variation three



different sizes of the several species of fish were examined whenever it was possible to obtain them. In selecting individuals for study it was assumed that the larger fish were the older.

Perca flavescens (Mitchill)

The first fish selected for a detailed study was *Perca flavescens*, because of its abundance in Douglas Lake and the ease with which it might be obtained, since numbers of individuals might be found in almost any sizes desired along the beach after a high wind.

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Fishes of these seemingly desirable sizes, that is, 150 mm. or more in length; 100 to 125 mm. in length; and less than 100 mm. in length were collected along the beach and brought to the laboratory. All were preserved in 5% formalin.

All the scales from the three bands on the three different individuals, each representing one of the three different lengths, were mounted and the data taken. From these data curves were plotted and it was found that the resultant curve for Band 1 and Band 2 were very similar in shape, but that Band 3 was somewhat different. The curves for the three bands of one of these three perch are shown in figures 1, 2, and 3, and the entire data is given in

Figure 2
—— Right side
····· Left side

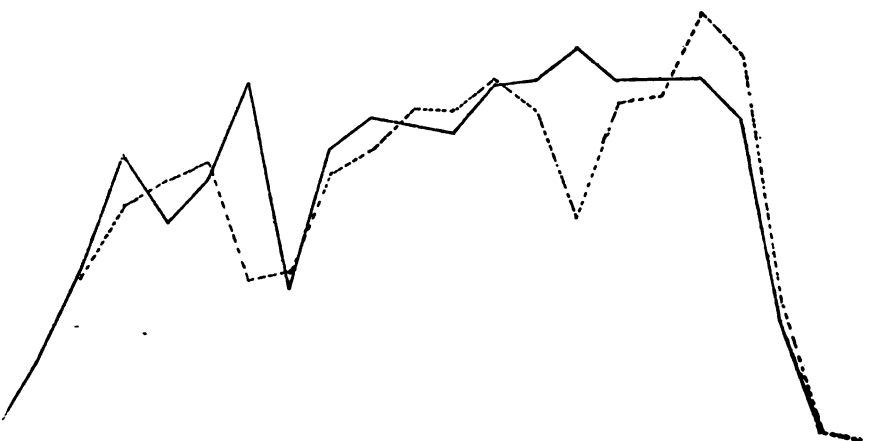

Table 1. If a detailed study of this data is made it will be found that the lowest points of the curves are represented by the extreme scales; that is, the scales from the median dorsal and median ventral lines. In view of the fact that these points seemed so constant for both sides of the fish and for all the fish examined, data was recorded only from the extreme dorsal and ventral scales and the scales from either the second or third row above and below

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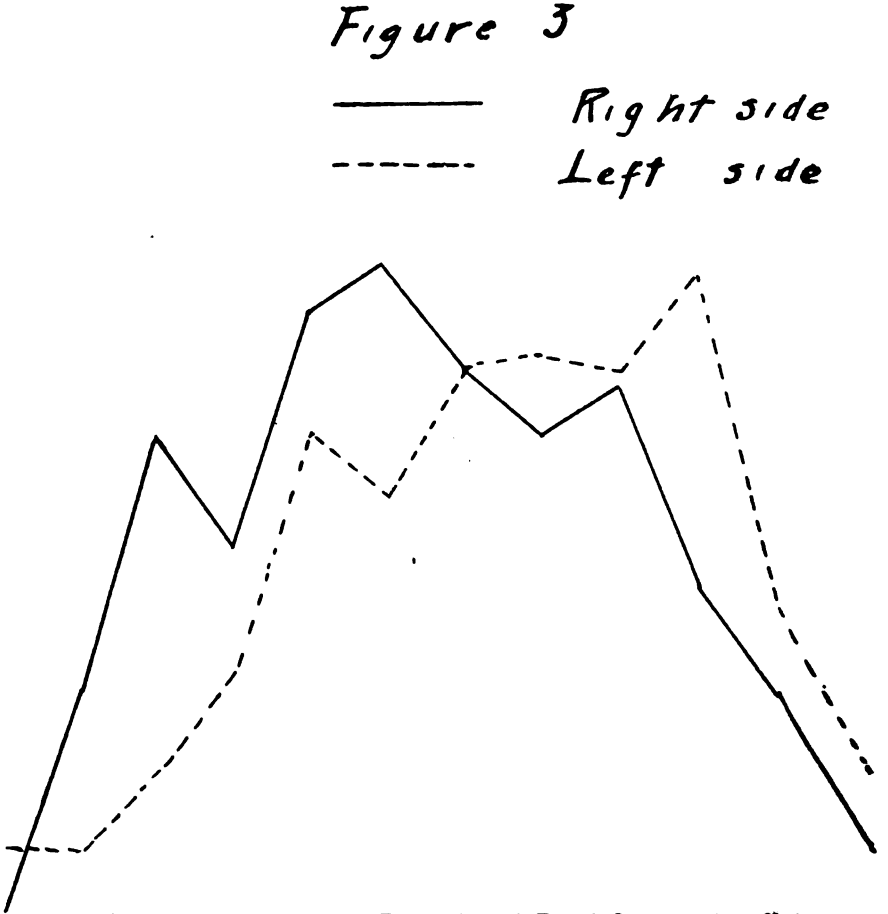
the lateral line in subsequent tables. In comparing the curves representing the various bands with curves for the corresponding bands on the opposite side of the fish they were found to be similar, which indicates that corresponding scales from opposite sides of a fish are rather uniform but not identical.

Figure 3



The similarity between Band 1 and Band 2 seemed sufficient to warrant the use of only one of these bands. Band 2 was discarded. This should not be taken to indicate that no scales were examined from the vicinity of Band 2 as scales were examined

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from all parts of the body but data was only recorded for Bands 1 and 3.

Table 1 contains the data collected in the detailed study of the three different sized *Perca flavescens*. It includes averaged data for every scale in the three typical bands. The data for each scale was collected separately and then the corresponding data for each of the several scales of the various series were averaged. The lengths of the three sizes of fishes were, Series 1, 242 mm.; Series 2, 200 mm.; Series 3, 90 mm. A detailed study of this table shows a very great similarity in scales from similar parts of the body. In studying scales from the various fishes it was always found that the scales in the row along the base of the various fins are not well developed and may even fail to show signs of teeth, radii, or lobes. This is very likely due to the position which they occupy and it appears that they are entirely normal for the region which they represent. Many of these scales are covered with skin. Ctenoid scales are in general not heavily covered with skin. This fact may account for the presence of cycloid scales along the base of the fins. It might be contended that the dorsal and ventral scales examined in this work were such scales. This is probably not the case, however, as almost without exception the scales from Band 1 showed radii and lobes. Also a thorough examination was made of all the scales in front of the dorsal fin and they were found to be very much alike. In the scales from the tail region or from Band 3, with very few exceptions, all were highly ctenoid and had both radii and lobes. This brings out a point in regard to the scales of the caudal region. In every *Perca flavescens* examined the scales of that region were found to be highly ctenoid. This was also true of most of the other fishes examined. It shows very well in *Micropterus salmoides* (Lacepede) and *Micropterus dolomieu* Lacepede which have scales that are cycloid or very weakly ctenoid on the anterior part of the body while the posterior part of the body is covered with scales that are heavily ctenoid. In *Perca flavescens* it may be said that, in general, the caudal region is more heavily ctenoid than the anterior region. The biological significance of this fact was not ascertained. Additional protection from rear attacks or for use in connection with spawning (like the pearl

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organs in the Cyprinids) might be suggested. Again it may have no significance whatever.

If Table 2 is examined it will be found that there are many differences between the scales of the larger fish and those of the smaller fish; also that there are many similarities. It is very noticeable that the number of teeth increase with the size of the fish. Scales from the fish of the larger size have the greater number of teeth, these being twice as numerous as the maximum number in the scales from the smaller fish, in some cases. The teeth on the smaller scales are very pronounced, however, even though some of the scales are very small. This variation in the number of teeth on the scales of the different sized *Perca flavescens* seems to be one of the most certain characters by which the scales from a large fish may be separated from those of a smaller fish. The radii and lobes are much more constant. However, there is a tendency for the smaller scales to have a greater number of lobes than the larger ones. This is correlated with the fact that in nearly all the scales from the smaller fish the radii are usually all complete, while in the scales from the larger fish many of the radii are incomplete. This is also a very sure way to distinguish a small fish from a larger one. The more complete and more numerous radii of the scales of the smaller fish is interesting in view of the fact that the radii in the scales of *Osteoglossidæ* and other more primitive fish are very numerous, complete, and often cross the entire scale, while there is a tendency toward the reduction of the radii in the scales of the higher fish. The scales from the various parts of the body of the fish are variously shaped. Those from the sides are rather broad and the teeth are stout in appearance. Scales from the dorsal and ventral parts of the body vary considerably in shape. Most of them are more or less lop-sided. The scales along the sides near the lateral line decrease in size from the anterior end of the body back toward the caudal end. On the cheek of *Perca flavescens* the scales are not toothed. From a smaller fish the scales do not appear so coarse and the teeth are somewhat more frail. The lobes in the older scales are much more pronounced, the basal end being deeply notched and the individual lobes being more elongated. In the smaller fish the lobes of the scales resemble a series of small semi-

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circles. In other features the scales appear much alike. Scales from the side of the fish appear more or less rounded and somewhat flattened on the apical and basal ends. A description of a typical *Perca flavescens* scale covering scales from all parts of the body would include the following characteristics: The shape of the scales are from oblong to ovoid with from three (some scales along the base of the various fins without teeth) to seventy-seven teeth on the outer margin of the apical area, varying in size with the size of the individual fish the scales are taken from. The apical area upon which these teeth are set is built up of columnar-like structures. The radii are all basal and extend out from the nucleus, which is situated about one-third of the distance from the top of the apical area to the base of the basal lobes, through the basal area in the form of spokes varying in number from one to twelve. Many circuli extend as semi-circles through the outer part of the basal area of the scale. In the outer part of the scale these circuli are interrupted by the apical area while in the central area they are not but extend around the nucleus as small circles. This is much more pronounced in the scales from the smaller fish than those from the larger fish. In the smaller fish the circuli are all beaded. In the larger fish the circuli in the center are not beaded, evidently having lost them in the course of development. The circuli are beaded in the outer part of the older scales. These points concerning beading are what might be expected since primitive fish have beaded circuli.

Stizostedion vitreum (Mitchill)

The only examination of the scales of *Stizostedion vitreum* was made on the scales of large specimens taken from Carp Creek.

A comparison of the scales of *Perca flavescens* and *Stizostedion vitreum* show dissimilarities in a great many respects. In *Stizostedion vitreum* the scales are more heavily toothed in all parts of the body. The dorsal and ventral scales are also heavily toothed which is not the case in *Perca flavescens*. Also the scales of *Stizostedion vitreum* usually have a decided flange between the apical and basal areas.

In a detailed study of the scales of *Stizostedion vitreum* all the circuli are found to be heavily beaded looking in part as if the

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circuli were broken in pieces. Also the circuli do not extend through the apical area even in the central part. Whether this would hold for smaller specimens is not known. An examination of scales from all parts of the body showed no regional differences from *Perca flavescens* other than the dorsal and ventral regions being heavily toothed.

Table 3 gives the complete data for the only two specimens of *Stizostedion vitreum* examined.

Boleosma nigrum (Rafinesque)

As *Boleosoma nigrum* is a very common fish in the shoals and along the rocky shore of Douglas Lake, large numbers were available for study.

One of the points in which it differs from the rest of the *Percidæ* is in the fact that the breast and the dorsal parts in front of the dorsal fin are scaleless. Forbes and Richardson* hint that probably more northward the specimens of this fish are more scaly than southward. None of the specimens taken from Douglas Lake, however, were found to have scales on the breast and cheeks, and only a few were found that had scales on the back. In tabulating the data for Table 4 the absence of scales on the breast and dorsal parts of the fish made a slight change in the scales taken necessary. In taking the dorsal scale the data was recorded for the very top scale near the front of the dorsal fin. The ventral scales were taken from the ventral line just back of the ventral fins. This plan was followed throughout regardless of whether the breast was scaled or not.

Boleosoma nigrum being one of the more highly specialized *Percidæ* it would be expected to possess scales of a higher type. This is exactly what is found when the scales are studied. Table 4 shows that all the scales are distinctly ctenoid. The teeth are very prominent and although the scale is rather fragile in appearance it is well developed and the teeth look very formidable.

The data also show that the radii are somewhat more numerous than in either *Perca flavescens* or *Stizostedion vitreum*. When compared with the scales from very small *Perca flavescens* they

*"The Fishes of Illinois," by the Natural History Survey of Illinois. Volume III pp. 297, paragraph 2.

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seem to be somewhat alike although the young *Perca flavescens* scales are much more immature in looks. The lobes appear quite alike. The circuli in *Boleosoma nigrum* as would be expected in a highly specialized fish are plain, appearing as a number of plain line semi-circles and not extending through the apical area.

Centrarchidæ

In a further study of scale characteristics the *Centrarchidæ* were selected as a group to be studied. They are very common in Douglas Lake being represented by five different species.

Fifteen different specimens were examined in every case possible. Some, however, are represented by fewer specimens as they were unable to be obtained in greater numbers. Unlike *Perca flavescens* most of the specimens were taken in nets. A few, however, were collected along the beach. In the study of the *Centrarchidæ* the same plan was followed as in the study of the *Percidæ*. Although only data for eight scales were recorded a careful study was made of scales from all parts of the body. The species studied were: *Ambloplites rupestris* (Rafinesque), *Eupomotis gibbosus* (Linnæus), *Lepomis pallidus* (Mitchill), *Micropterus dolomieu* (Lacépède), and *Micropterus salmoides* (Lacépède).

The study of the scales of the *Centrarchidæ* is a varied and interesting one. A typical scale of one of these fish is very much like one of *Perca flavescens* in appearance. The circuli are beaded and do not extend through the apical area except in the very young specimens. In such specimens the circuli passing through the apical area are not beaded. Also in scales from a young *Centrarchidæ* the apical area is entirely covered with circuli. One of the marks that will help to distinguish a *Percidæ* scale from one of a *Centrarchidæ* is that the apical area in a *Percidæ* scale forms a more obtuse angle than in the *Centrarchidæ* scales.

In the *Centrarchidæ* the dorsal and ventral scales do not have the same characteristics as the ventral and dorsal scales of *Perca flavescens*. In *Ambloplites rupestris* the shoulder including the parts of the fish in front of a line drawn from about the fourth dorsal spine to the tip of the pectoral fin and on the ventral surface of the body in front of a line drawn from the base of the ventral fins to the tip of the pectorals, is without ctenoid scales. The scales

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from these parts of the fishes body are either entirely without teeth or with only traces of teeth. The boundry of these regions vary more or less but in general the above statement will about cover it. Occasionally a ctenoid scale will be found to occur in these regions but it is rather uncommon. In *Eupomotis gibbosus* and *Lepomis pallidus* the dorsal regions correspond with those in *Ambloplites rupestris* as may be seen by the data. The ventral regions are, however, entirely different. In both *Eupomotis gibbosus* and *Lepomis pallidus* the scales from the very lowest ventral parts are all ctenoid. Further up near the opercular flap only traces of teeth are found. In *Ambloplites rupestris* the ventral part of the fish near Band 1 is found to be covered with scales that are distinctly cycloid or almost always so.

In all the *Centrarchidæ* studied the scales from the caudal region were found to be highly ctenoid. The scales along the base of the fins are usually long, irregular in shape, and without teeth, radii, and lobes.

Ambloplites rupestris

The specimens examined varied in length from 17 mm. to 215 mm. The data (Table 7) show how distinctly cycloid the scales from the region of Band 1 are. The data for the scales from Band 3 show that the scales from that region are very decidedly ctenoid. In shape, the scales of *Ambloplites rupestris* are much like a scale from any of the other *Centrarchidæ*.

Lepomis pallidus

The specimens examined were from 52 mm. to 125 mm. in length. Table 9 shows that *Lepomis pallidus* differs from *Ambloplites rupestris* in that the scales from the ventral part of the fish in front of the ventral fins, are ctenoid while in *Ambloplites rupestris* they are cycloid. The scales from the caudal regions of these two fish are quite similar.

Eupomotis gibbosus

The specimens examined varied from 62 mm. to 135 mm. in length. Table 8 shows that the scales are ctenoid except in the region of the shoulder. The ventral scales on Band 1 are ctenoid but farther up the sides near the operculum only traces of teeth are

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found. The scales from the ventral region show more or less of a tendency to be flanged, that is, the region in which the apical and basal areas meet is wider than the parts of the scale on either side of it.

Micropterus salmoides and Micropterus dolomieu

The scales from *Micropterus salmoides* and *Micropterus dolomieu* will be discussed together as they are both typical *Centrarchidæ* scales and resemble the other *Centrarchidæ* discussed very much. Further, their own relations are better discussed when they are considered together. The cycloid regions in these fish are quite similar. It is also very much more extensive than in any of the other *Centrarchidæ* studied. These scales from all the anterior part of the body regardless of their position are cycloid or at most very weakly ctenoid. The posterior part of the fish is covered with scales that are highly ctenoid. If scales are selected and examined from the posterior end of the fish forward they are found to gradually pass from ctenoid to cycloid. Only a few fish were available for the work so that a more detailed study is lacking. From the data at hand it appears that the ctenoid scales extend as far forward as about the third or fourth ray of the dorsal fin. In the study of a large number of scales from the different specimens it was found that *Micropterus salmoides* shows a tendency to be more heavily ctenoid than does *Micropterus dolomieu*. A description of a typical scale will suffice for either of these two fish. In the younger specimens the circuli extend around the nucleus, passing through the apical area, and are beaded, while in scales from the adults the circuli extending through the apical area are not so numerous and also the circuli in the central part of the scale are not beaded. The outer circuli in the adult scales are very much broken and show very distinctly a tendency to form teeth.

Percopsis guttatus (Agassiz)

The study of the *Percopsidæ* was made upon *Percopsis guttatus*. They varied in length from 75 mm. to 87 mm. This fish has a scale which is very different from either the *Percidæ* or *Centrarchidæ*. It has no radii or lobes. The nuclear area is located far up

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near the apical end. Also the plain circuli in the central area near the nucleus extends through both the apical and basal areas. These circuli appear to be broken in places. Farther out they are interrupted by the very small apical area.

The teeth are small but very pronounced. They are very different from those on the scales of *Percidæ* or *Centrarchidæ* in that the apical area is not composed of columnar-like structures. Although the teeth are well formed they appear rather weak due to the fact that the apical area lacks the columnar-like structures. The number of teeth on each side scale varies from 2 to 22 and as in both the *Percidæ* and the *Centrarchidæ* the posterior end of the body is more heavily ctenoid than the anterior end. The nuclear area is also nearer the top of the apical area than in the *Percidæ* and *Centrarchidæ*.

In *Percopsis guttatus* the dorsal part of the body in front of the dorsal fin is scaleless. The table does not include this region. The first data recorded represents a scale from the second row above the lateral line in every case. Table 5 contains the data collected on *Percopsis guttatus*.

Esox Lucius (Linnæus)

The scales of *Esox lucius* show the characters of a more primitive fish when compared with those of one of the more highly specialized fish examined. The scales are entirely without teeth. The number of radii found were from one to four and the number of lobes varied from one to three. In many of the scales the lobes were found to be separated from each other and the rest of the scale, in many cases, over half way to the nuclear area.

The circuli are plain and somewhat irregular. They extend as circles through both the apical and basal areas. In the center near the nucleus they become irregular in shape. In many of the scales examined there were irregularities in the upper portion of the apical area which may be an indication of teeth forming. Table 6 gives the data collected on the two sizes of *Esox lucius*.

SUMMARY

It has been very recently that scale characters have been considered seriously as a method of distinguishing between different

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fishes. Undoubtedly external features are a quicker method by which to identify a fish, but if only a small portion of the fish is available then the method of identification by scale characteristics becomes very valuable, if these characteristics are stable. That they are stable seems almost conclusively proven from the data at hand. A few of the essential differences in the scale characteristics of the fish studied may be enumerated.

In *Perca flavescens* the dorsal row of scales in front of the dorsal fin, and the ventral row of scales in front of the ventral fins, are not ctenoid. The rest of the dorsal patch which may be designated as all the scales in front of a line drawn from the anterior end of the dorsal fin to the corner of the operculum, and all of the ventral patch which may be designated as all the scales included in the region in front of a line drawn from the base of the ventral fins to the corner of the operculum, is covered with scales which are toothed very heavily. This is not true of any other fish studied. The scales of *Boleosoma nigrum* are ctenoid on all parts of the body. If a scale of a small *Perca flavescens* is compared with a scale from *Boleosoma nigrum* they can be readily distinguished from each other regardless of the sizes of the fishes that they are selected from. The scale from a *Boleosoma nigrum* is a mature looking scale. It is well formed and looks as formidable as a very mature scale from a *Perca flavescens*. The scales from a small *Perca flavescens* have a rather immature appearance and are easily distinguished as scales from an immature fish. In such scales the columnar structures are not well formed and altogether the scale looks frail in comparison with one from *Boleosoma nigrum*. Also *Boleosoma nigrum* lack the scales on the breast.

In the *Centrarchids* studied the whole shoulder patch is without ctenoid scales. The ventral patch is different. In *Lepomis pallidus* and *Eupomotis gibbosus* the ventral patch is covered with ctenoid scales, the teeth being more or less lacking up near the operculum. The scales of *Ambloplites rupestris* are cycloid on all the ventral patch distinguishing it at once from either *Lepomis pallidus* or *Eupomotis gibbosus*.

Micropterus dolomieu and *Micropterus salmoides* are distinguished from the *Percidæ* and *Centrarchidæ* studied by the absence

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of ctenoid scales on the anterior part of the body. This region with only cycloid scales extends back to about the third or fourth ray of the dorsal fin.

Esox lucius is easily distinguished from the rest of the fish studied by the body being entirely covered with cycloid scales. Also the lobes are distinctly separated from one another by the radii, almost one-half of the distance to the nucleus.

Percopsis guttatus scales may be identified by the absence of any columnar-like structures in the apical area.

The work so far as it has been carried out and so far as the data can be interpreted seems very satisfactory. Many striking similarities are seen between the scales of different fishes from the same families. Also many differences are evident in the scales from fishes of the various families. In every case, however, the scales of the primitive fish have shown characteristics of primitive scales, and the scales of the specialized fishes have shown characteristics of highly developed scales.

Scale characters as a means of identification would probably be very little affected by regeneration as the percentage of regenerated scales is small. The percentage of regeneration in all the scales of a single specimen of *Amblophtes rupestris* about 200 mm. long was 12%.

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

A RELIABLE METHOD FOR OBTAINING AMŒBA FOR CLASS USE

By Charles A. Kofoid

It is a common experience of the teacher in elementary biology to find that his supply of *Amœba* for class use is uncertain in source, variable in amount, and not infrequently fails at the appointed time, or is so scanty that pupil and teacher both find more experience in searching for the elusive creatures than in profitable observation. Those who have available an *Oscillaria* rich margin at some outlet of sewer or of a drain where a fairly permanent greenish-black felt of this alga is present, can often secure an abundant supply of large *Amœba proteus*. Decaying *Ceratophyllum* from pond or lake waters also yields this *Amœba* in abundance for a brief period in the process of decomposition in the laboratory aquarium. A permanent *Amœba* culture of this species might be arranged and regularly kept up in a culture house with supply of spring water suitably enriched. A culture of this sort of an apparently as yet undescribed *Amœba* was maintained at Lincoln, Nebraska, by Miss Powers and supplied widely to the laboratories of the country from Atlantic to Pacific for several years, but was unfortunately destroyed by purification treatment of the city water supply.

In our laboratories at the University of California we have for several years applied the principle of pure mixed cultures in a crude way to the production of amœbas in quantity for class use with such success that we offer it as one solution of the problem of supply for large classes where the elimination of loss of time in searching for the animals on the slide is desirable. The amœba

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we have cultivated in a small soil amœba, *Nægleria gruberi* (Schardinger) originally described by Schardinger (1899) from the human intestine in a case of diarrhea. It is improbable, in view of the use of pure culture methods in his work, that his culture was contaminated from dust or water; though it is not impossible. The amœba in reality may have been recovered from the fæces after transit in an encysted condition through the intestine of the patient having been introduced in food or water. This is apparently the same amœba which Wherry (1913) later isolated from the water supply of Oakland, California.

There are certain disadvantages in its use. It is very small, about 9 to 30 microns, is not very mobile at room temperatures, lacks prominent pseudopodia in most cases, and perhaps worst of all, enflagellates on slight provocation, often at a very inopportune time, and quite without regard to class schedules. The compensating advantages are (1) the great numbers easily obtained, ten or a dozen in a single high-power field, and thus the entire elimination of the necessity of search for the animals; (2) the occurrence of cysts, amœboid, and flagellate stages and of both exogenous and endogenous budding; (3) ease and certainty (in our experience) of securing the cultures; and (4) ease with which slides of the various stages may be prepared. A full account of its life-history, as far as known, is now in press in the "University of California Publications in Zoology" by Dr. Charlie W. Wilson (1915) now of Mills College, Oakland, California.

The methods of culture which we employ for laboratory supply are as follows: We use as culture dishes enameled instrument trays 6 by 10 inches in size which fit readily in the horizontal autoclave, and accomodate each over fifty 22mm. floating cover glasses. As nutrient culture medium we use the filtered fluid from a mixture of 50 grams each of lettuce leaves, horse manure, soda cracker, and garden soil boiled for half an hour in a liter of creek water or untreated tap water. This fluid is sterilized for 30 minutes in the autoclave and when thoroughly cool is shaken up with a small quantity of soil and the water poured in the previously sterilized pan to the depth of 2 centimeters and covered with a glass plate. The pans should not be placed in the direct sunlight.

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Clean cover glasses are floated carefully by dropping them on the surface of the culture fluid and are best removed by bent cover glass forceps. Soil bacteria and the soil amœbas appear within a few hours, and multiply rapidly, and are at a maximum in about four days after the culture is started, the period varying according to a number of factors such as temperature, richness of the medium, and certain unknown chemical conditions. The medium should not be too rich for best results and may be diluted to one-half or less the strength above indicated by use of a larger proportion of water. The numbers of amœbas are less in the poorer cultures, but there is danger of excessive bacterial growth and fermentation if the medium is too rich.

The amœbas rise to the surface and adhere in both amœboid and encysted stages to the under surface of the cover glass in astounding numbers. For class use it is only necessary to make certain of infected soil by a trial culture, and of the time interval between seeding the culture and the period of maximum abundance of amœbas under the conditions of temperature, stock of culture medium in use, etc. It is desirable to use a uniform, or the same stock of culture medium for both the preliminary test and final culture. When these factors are determined it is safe to proceed and to rely upon the culture to yield the amœbas as planned. It is possible to plant new cover glasses in the culture after removing the first supply and within 24 hours to have a new lot available. The cultures run down rapidly at first and then slowly for weeks or months after their brief maximum.

We have found it advisable to keep a stock in culture and to seed from it by adding a cubic centimeter of old culture fluid to the unseeded new medium instead of using the original soil material. Such a culture has now been continued in our laboratory for over thirty months.

The conditions which stimulate enflagellation are apparently access of oxygen and new food supply. For this reason cover glasses should be used promptly on removal. They not infrequently yield enflagellating amœbas within an hour and usually the whole culture on the cover glass follows suit. Encystment is very common and occurs both with abundant and with scanty food supply. Stained

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preparations are readily made of the amœboid and encysted stages by floating the cover glass with its attached amœbas on warm Schaudinn's fluid amœba side downwards for a few moments, and then staining by the customary procedure in Heidenhain's iron hæmatoxylin. Amœboid, budding and encysted stages are abundant in such preparations and enflagellating, exflagellating stages and the flagellate stage itself are all to be found by careful search but are obviously more difficult to obtain in this way since they do not adhere habitually to the substrate.

Many of the amœbas are in stages of cell division, and many cysts are binucleate simulating sexual phases. Care is essential in interpreting these stages as sexual phenomena, which are as yet not proven to occur in this amœba. Chromidial extrusion simulating maturation phenomena also occurs in the cysts and many puzzling nuclear conditions, probably degenerative or involution phenomena, will be found in stained preparations from cultures, especially in the period of the decline from the maximum numbers.

Cultures easily become contaminated and ciliates from the soil are usually inoculated with the amœbas. For this reason it is wise, if one wishes to keep a permanent culture going, to isolate a ciliate-free cover glass for seeding the permanent culture.

Our experience leads us to believe this culture method adds a valuable resource to our biological laboratory and increases our means of control of an abundant supply of this very important type essential both for instruction and research.

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October 22, 1915.

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NOTES ON METHODS OF LABORATORY TECHNIQUE FROM THE ZOOLOGICAL LABORATORY, UNIVERSITY OF MICHIGAN

The writer presents the following methods of laboratory technique knowing full well that little originality can be claimed for them but with the feeling that they have been sufficiently tried to prove their worth.

Neutral red (Neutral Roth) as an indicator of the reaction of the digestive fluids of Protozoa.—That neutral red may be used as an indicator of the reactions of the digestive fluids of Protozoa has been known for a considerable time but its use as such is not as general as it should be. For this purpose freshly made solutions of the dye in the proportion of 1 : 10000 to 1 : 100000 in distilled water should be used. A few drops of the solution should be drawn under the cover-glass or one or two drops of it should be added to the water on the slide containing Protozoa before placing the cover-glass. Examination should be made at once because the reaction is secured within a few minutes. In the presence of alkalies neutral red becomes yellow while in the presence of acids it becomes red. Used in this manner the stain does not injure the Protozoa appreciably. This test is readily made even by a beginning student and it is easily interpreted by him. In this laboratory it has been used in the study of Paramecium in order to introduce a little idea of the physiology of Protozoa. It should be further stated that after the digestive fluid in the food vacuoles becomes well stained the mouth and gullet are more readily seen than in the untreated specimens.

To demonstrate the difference in function of the skin glands of the frog.—It has been the usual experience that ordinary methods of fixation and staining of the skin of the frog do not yield preparations which readily show differences in function of the poison and mucus glands. Hæmatoxylin and eosin usually reveal only structural differences not differences in secretions elaborated. After numerous trials the following method was found to give best results: Pieces of skin including the dorso-lateral dermal plicæ are removed from a pithed frog, tied to a slide flesh side out and fixed over night in Bouin's picro-aceto-formol mixture. Ten micron sections from this material are stained for a few minutes in a 0.1%

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solution of thionin in distilled water, washed for a few seconds to a minute in distilled water, and briefly stained in a 0.5% solution of erythrosin. The section must then be dehydrated rapidly, cleared in xylol and mounted in balsam. By this method all nuclei are stained blue, the contents of the small glands and the granules within the cells of these glands are stained blue to reddish-purple. Since thionin is a specific mucin stain these glands are shown to be mucus glands. While the nuclei of the cells of the large glands are stained blue the cells are either unstained or are pink; the secretion which appears in the lumen of the gland as globules of varying sizes is stained a brilliant reddish pink or red tinged with yellow. Thus these glands are shown at once to be different in function from the mucus glands, and the inference is that they are poison glands. With such striking colorations the two kinds of glands are distinguished at a glance. By this method the muscles surrounding the glands are stained pink, the stratum compactum another shade of pink, blood cells pink or coppery red with blue nuclei. Boundaries of epidermal cells are clearly shown.

Fixation in Gilson's fluid followed by these stains gives fair results but not as good as by the method outlined above. Unfortunately sections stained by this method are not colored uniformly from end to end, nor are different slides stained alike, nor is it possible to turn out slides by wholesale when using these methods because each slide must have individual treatment. Slides so stained, however, show many features not well differentiated by hæmatoxylin stains in combination with other stains.

In the absence of thionin toluidin blue (Toluidin-Blau) used in the same fashion gives almost the same results. Kresylectviolett has not been tried on this tissue but it should be pointed out that it is an excellent mucin stain.

A method for staining the cephalic glands of immature trematodes.—In an attempt to work out the relations of the cephalic glands to their ducts in some minute immature trematodes a number of aniline dyes, carmine, hæmatoxylin stains were tried. The material had been fixed in formalin of unknown strength. Hæmatoxylin which was first tried for toto preparations and sections gave altogether erroneous ideas of relationships of the glands to the

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ducts and wrong ideas of the ducts because of over-staining and too diffuse staining. Carmines were hardly more successful. Out of a number of aniline dyes solutions of thionin and of toluidin blue, ca 1% in distilled water, gave excellent results either for toto mounts or for sections. Whole specimens or sections were stained about ten minutes in either of these stains, differentiated by washing in distilled water, dehydrated and finally cleared in xylol and mounted in balsam. The length of time required for differentiation of the stain in the whole worms was several minutes while a few seconds or at most a minute sufficed for the sections. Sections were sometimes given a second stain with erythrosin which served to stain muscles but did not aid in the study of the glands and the ducts. The contents of the glands and of their ducts when stained with thionin or toluidin blue are blue or purple in color and are seen to consist of great numbers of small globules. In this case the indications are that this material is mucin. The walls of the glands and ducts remain unstained. As a result of this fact two ducts which lie very closely together and which appear as one when stained with hæmatoxylin or carmine are shown as separate structures. Certain other structures are shown fairly well. The method seems to be inapplicable to toto preparations of large trematodes.

To fasten the wax to the bottoms of dissecting pans.—The usual method of fastening the wax in a dissecting pan is to have strips of tin or other metal soldered or riveted to the ends or to the bottom of the pan and so projecting that the melted wax will run under the strips and hardening thus the mass is held in place. This method does very well for tin or copper pans but if it is desired to use enameled pans it is not satisfactory. A method that is applicable to any kind of a pan is to use 'BB' shot or bits of granulated lead or zinc to weight down the wax. At the time of pouring in the melted wax the shot or the bits of granulated metal are placed in the corners of the pan. When cooled the weight of the metal holds the wax in place. In case the wax becomes loosened it can be readily removed from the pan, melted and repoured. When metal strips are used in the old method the wax must be melted in the pan and there is danger of opening up the seams in soldered pans.

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Celluloid-acetone cement.—A very useful laboratory cement may be made by adding scraps of celluloid to acetone in which it quickly dissolves. Experience will soon teach the proper proportions. Do not make it too thick. Since acetone evaporates quickly the cement must be kept covered and the work must be rapidly done. In our laboratory it finds many uses, e. g., in mending small broken bones, cementing glass to glass or to many other substances, where a waterproof cement is desirable. In mending small broken bones with this cement it is advisable to run some of the cement into the cavity, put pieces together, cover with a thin layer of the cement on the outside. If a strong joint is necessary chop up some cotton into short bits and work it into the cement as it is used. For the mending of bones the transparent celluloid should be used to make the cement but for other purposes it may be desirable to use colored celluloid. The writer has seen this cement so skillfully used on broken bones that very close scrutiny was required to discover the fracture. Since this cement is dissolved or at least softened by strong alcohols it cannot be used to mend things that are to be stored in alcohol.

To render visible lines milled on laboratory apparatus.—The millings on apparatus that has been in use for some time usually becomes indistinct. To render it again visible polish the surface with a good metal polish and then fill the milled lines and figures with 'Shinola' shoe polish, allow to dry and then rub off that portion which remains on the plain surface. The lines will be filled with the blacking. This material being neutral in reaction will not injure the metal.

To revive or to keep going cultures of Ameba.—Ameba cultures made after the method of Jennings, i. e., by allowing plant materials collected from ponds or streams to decay in pond or stream water usually begin to fail soon after they have begun to yield Ameba in abundance. Such cultures may be kept going or, even after all amebæ have apparently disappeared, they may be made to yield in abundance if a small quantity of sterile hay be added at intervals of a few days. Probably any clean hay would do as well but if sterile hay is used there is no danger of introducing other encysted Protozoa.

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ENTOMOLOGICAL NOTES

Bombycine Moths.—The National Academy of Sciences (1914) has recently published, as First Memoir of Volume XII, Part III of the "Monograph of the Bombycine Moths of North America", by A. S. Packard. The manuscript, left unfinished at the death of the author, was edited by Prof. T. D. A. Cockerell, assisted by a number of other workers. This part of the Monograph includes the families *Ceratocampidæ* (exclusive of *Ceratocampinæ*) *Saturniidæ*, *Hemileucidæ* and *Brahmæidæ*. This large volume contains a systematic discussion of each of the North American species and important data are given for immature stages. Tables for the separation of genera and species are included. One hundred thirteen plates accompany the text matter, of which thirty-four are wholly or in part in color, representing primarily the larval stages of many species. Students of *Lepidopera* will find this work of great interest.

Air Stores of Aquatic Insects.—Ege ('15, Zeit. f. Allgemeine Physiol., 17:81-124) has investigated the respiratory function of the air stores carried by *Corixidæ*, *Dystiscidæ*, and *Notonectidæ*. These air stores are found to have two distinct functions: (1) They have a hydrostatic function, thus playing a very important rôle in connection with the equilibrium of the animal and its relation to the surface of the water. (2) They are of respiratory importance and related to respiration in two ways: (a) they function in ventilating the tracheal system when the animal is submerged, and (b) they constitute a mechanism by means of which the insect is able to utilize the dissolved oxygen of the water.

Trichopterous Larvæ.—Krafka (15, Can. Ent., 47: 217-225) has made a study of trichopterous larvæ and presents a key to the families which will, no doubt, prove of great service in the identification of the immature forms. The key also leads to the identification of some of the principal subfamilies. Thirty-seven figures of structural detail of larvæ are included in the paper.

Germ Cells in Hymenoptera.—Hegner ('15, Journ. Morph., 26: 495-535) reports the results of studies on the protoplasmic differentiation in the oocytes of some of the *Hymenoptera*. The ovariole of *Apis mellifica* has four regions: terminal filament, rosette

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region, differentiation zone, and posterior linear series of oocytes separated by nurse cells. Each rosette group arises from a single oogonium and the component cells are held together by persistent spindle fibers from preceding mitoses. Oocytes and nurse cells arise from oogonia, the descendants of which can be determined by the presence of certain deeply staining rings between the cells. Granules, apparently mitochondrial in nature, appear near the nuclei of oocytes, later becoming distributed throughout the egg cytoplasm. In *Camponotus herculeanus* var. *pennsylvanica,* each ovariole consists of a terminal filament, terminal chamber, growth zone devoid of "bacteria-like rods" and posterior, linear series of oocytes accompanied by nurse cells, the former being surrounded and subsequently invaded by the bacteria-like bodies. Secondary nuclei, the origin and fate of which are in doubt, appear near the oocyte nucleus, increase in number, ultimately surround the germinal vesicle, and finally become distributed throughout the oocyte, particularly in the vicinity of the follicular epithelium. In *Copidosoma gelechiæ, Apanteles glomeratus,* and *Andricus punctatus* the changes in the oocyte nuclei are very similar, consisting essentially of early chromosome formation, paired fusion of chromosomes, arrangement on an asterless spindle, and condensation. The oocytes of *Diastrophus nebulosus* contain a chromatin body which probably results from chromosome condensation. In certain growth stages of *Rhodites ignota* large numbers of secondary nuclei appear, originating, apparently, from certain peripheral granules which stain like chromatin.

New Order of Insects.—Crampton ('15, Ent. News, 26: 337-350) has studied the morphology and systematic position of the remarkable annectent form, *Grylloblatta campodeiformis,* which was recently described by Walker from specimens found in Canada and placed by him in a new orthopteran family, *Grylloblattidæ.* Crampton finds that the structural features of this species include a combination of characters found in *Dermaptera, Isoptera* and *Gryllidæ* and that there are sufficient grounds for considering *Grylloblattidæ* a distinct order, the *Notoptera.* This new order occupies a position intermediate between the *Dermaptera* and *Isoptera* and seems to

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include the "nearest living representatives of the common ancestors of the Gryllidæ and 'Locustidæ' (Tettigonidæ)."

Luminous organs.—Wheeler and Williams ('15, Psyche, 22: 36-43) find that in the New Zealand glow-worm, *Bolitophila luminosa,* the four elongated Malpighian tubules, which extend to the posterior region of the body, are differentiated into two parts: (1) the long, proximal portions, which retain the primitive excretory function of these organs; (2) the short, distal, dilated tips, closely applied to the intestine, which appear as four curved, luminous rods in the living larva and constitute the photogenetic organ. Distinct structural differences occur in the two differentiated parts. In no other insect are the Malpighian tubules known to have a photogenetic function, although they sometimes acquire functions other than that of excretion, as for example, the production of silk in certain *Neuroptera* and *Coleoptera.*

Wilt Disease.—Glaser ('15, Journ. Agr. Research, 4: 101-128) finds that the wilt of gypsy-moth caterpillars is a true infectious disease which occurs all over the gypsy-moth territory. Epidemics occur only in regions heavily infested with gypsy-moth and climatic conditions appear to have an important relation to the disease in the field. Certain imported parasites may be important factors in the distribution of the wilt. There is no direct evidence that the disease is transmitted from one generation to another. Infection naturally occurs through the mouth by means of the food. The disease is more prevalent among the older larvæ.

Olfactory Sense in Coleoptera.—McIndoo ('15, Biol. Bull., 28: 407-460) reports the results of an extended morphological and experimental study of olfaction in beetles. His results show that the antennæ do not bear any of the olfactory organs but certain pores, found on the peduncles of the elytra, on the dorsal surfaces of the wings, on the trochanters, tibiæ, sometimes on the femora and tarsi, and perhaps on the mouth appendages, are the true olfactory organs.

Sex recognition.—Sturtevant ('15, Journ. Animal Behavior, 5: 351-366) finds that, in *Drosophila,* the olfactory and tactile senses are probably concerned with sex recognition and that sight is not essential. The wings of the male function in the production of sexual excitement in the female. The characters of certain mu-

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tants—white eyes, vermilion eyes, yellow body color, and curved wings—seem to have no selective value. Evidence is presented in support of the view that neither sex exercises any "choice" in the selection of a mate but mating will occur when members of the two sexes, ready to mate, chance to find each other.

Notonectidæ.—Essenberg ('15, Journ. Animal Behavior, 5: 381-390) reports results of a study on the natural history and habits of *Notonectidæ*. The food is chiefly living or dead insects, and animals many times larger may be successfully attacked. Chemicals in solution have very little influence on backswimmers. They have a strong, positive phototaxis which increases with rise of temperature and with greater light intensity. They are positively rheotactic. The young resemble the adults in their behavior and instincts.

Habits of Water-striders.—Essenberg ('15, Journ. Animal Behavior, 5: 397-402) finds that *Gerris remiges* is positively thigmotactic, positively phototactic, positively rheotactic, and negatively geotactic. Sense of smell is apparently present; hearing is not well developed; and sight is very keen and efficient. No special choice is shown in the selection of food, any dead or living animal matter being consumed. Individuals may live for weeks or months without food. They are actively predaceous but never attack animals below the surface of the water. They possess the cleaning habit and may be engaged in this activity for considerable periods of time. Death feigning is common and may be produced by artificial stimulation.

Middle Membrane in Wings.—Marshall ('15, Annals Ent. Soc. Am. 8:201-216) finds that in *Platyphylax designatus (Trichoptera)* the structure, usually known as the middle membrane, is not a true membrane but is a thin layer of protoplasm which occupies a median position within the wing. During the development of the wing this median layer is not continuous but disappears and is reformed in the same position. The resulting layer is more membrane like than the one first formed.

Preservative for Insects.—Schulze, ['15, Entomological News, 26: 361 (abstract from Deut. ent Zeitschr., 1915, p. 204)] recom-

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mends the following fluid for the preservation of galls, coccids on plants, and larvæ for dissection: 200 cc. glycerine, 200 cc. distilled water, 1 gram crystallized carbolic acid.

This mixture is said to be serviceable for preservation in the tropics.

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NOTES ON OLIGOCHÆTA

Hæmonais.—Stephenson ('15, Trans. Roy. Soc. Edinburgh, 50:769-781) reports the discovery of a new species of *Hæmonais* in India. Heretofore, this genus was represented only by a single species from Switzerland. A complete account of the anatomy is given. In the sexually mature condition, the alimentary canal undergoes atrophy. The mouth remains but the lumen of the canal becomes indistinct and disappears in V, reappearing only posterior to XI. The posterior region is nearly normal in appearance but, in the zone of degeneration, the canal is merely band-like and its transverse dimensions are less than those of the ventral nerve cord.

Dero and Slavina.—Stephenson ('15, Trans. Roy. Soc. Edinburgh, 50:789-795) describes the anatomy of sexual individuals of *Dero limosa* and finds, in these animals, a curious degeneration of the digestive tract which has not been described in the Oligochæta except for *Hæmonais,* although a similar condition is known to occur in certain Polychæta. The anterior opening disappears, and the mouth cavity, pharynx, œsophagus, and intestine become so profoundly changed that they are represented only by a narrow cord for some distance. The lumen is absent anterior to XII. Apparently, the development of sexuality marks the end of the life of the individual. In this same paper, the hitherto unknown sexual organs of *Slavina* are described in the species *punjabensis.*

Bifurcation in Lumbricus.—Benham ('15, Trans. New Zealand Inst., 47:185-188) reports an interesting case of bifurcation in *Lumbricus rubellus.* The right side of the third clitellar somite gives rise to a posteriorly directed branch, about one-fifth as long as the body and containing fifteen somites. The clitellum is continued on the three basal somites of the branch and the tubercula pubertatis is continued along their outer side. The nerve cord and

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the nephridia extend to the end of the branch but the intestine is absent. Setæ are apparently absent on all somites of the branch except one. Bifurcations are not uncommon among earthworms but this record is unique because of the unusual anterior position of the branch, the great inequality of the two corresponding body parts, and the absence of the intestine in the branch.

Regeneration.—Hunt ('15, Am. Nat., 49: 495-503) has found that, in *Enchytræus albidus,* posterior regeneration occurs when the animal is cut at any level between "eight segments from the posterior end of the body and eight segments from the anterior end." From the caudal end up to the middle of the body, the rate of regeneration increases almost in direct proportion to the number of segments removed. Removal of the eight most posterior somites may cause regeneration of double tails. Regeneration occurs either in fresh-water or salt-water conditions and salinity apparently has little or no influence on the rate.

Earthworms.—Smith ('15, Bull. Ill. State Lab. Nat. Hist., 10: 551-559) describes two new varieties of earthworms from Illinois and presents a tabular key to the described species of earthworms known to occur in that state. The key is accompanied by additional data on the distribution and habits of the different species. The key will be very useful in the identification of the earthworms, not only of Illinois but also of the adjacent states.

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THE OLFACTORY SENSE IN INSECTS

This problem has been a favorite one for students for one hundred and fifty years, and very diverse conclusions have been reached. N. B. McIndoo has published a summary of these conclusions under the title "The Olfactory Sense of Insects" in the Smithsonian Miscellaneous Collections, Volume 63, No. 9.

The two principal views relative to the possible seat of the olfactory organs of insects are:—first, that they are located largely upon the antennæ and palpi; and secondly, that the vesicles discovered by Hicks and situated on the legs, wings, and other parts of the body are the true olfactory sense organs.

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While admitting that the removal of certain portions and experimenting with insects may aid in settling this question, the present writer is firmly convinced that observation of the normal activities of insects, and comparative study of the cytology of the various cells on the surface of the body of arthropods are more fundamental and important in determining how the function is distributed. In the opinion of the writer many arbitrary and misleading statements about the functions of surface structures of the insects are contained in the literature, largely because the authors were innocent of first-hand knowledge of the cytology of these structures. It is desired in this note to present a series of diagrammatic illustrations of the finer structure of some typical ectodermal cells taken from thousands of sections of insects.

Apparently *all* ectodermal cells are potentially sensory cells. The abundant branching of the nerve fibrils to the bases of these cells shown in Fig. 1 (Plate XVI) suggests this. The section here is of the pupa of the Tussock Moth and is perpendicular to the surface.

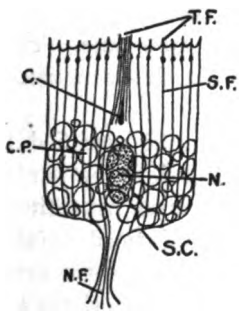

Fig. 2. Structure of typical sensory scale from Tussock Moth pupa. S.C.—sensory cell; N.—nucleus; N.F.—nerve fibril; C.P.—cyto-plastids; C.—centrosphere; S.F.—sensory fibrils; T.F.—terminals of sensory fibrils.

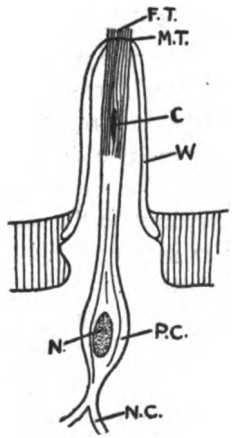

Fig. 3. Sensory Peg, *Vespa* Antenna. P.C.—peg cell; N.—nucleus; N.C.—nerve connection; W.—peg cell wall; C.—centrosphere; M.T.—membranous tip; F.T.—exposed fibril tips.

The typical internal structure of such a generalized sensory cell is shown diagrammatically in Fig. 2. In this type of cell there are two distinct types of fibrils having different origin. The fibrils marked (H) arise directly from small bodies in the outer end of

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the cell which in their turn arise from division of the centrosphere. These fibrils and their origin from the centrosphere suggest that they are homologous with the motile and nervous fibrils of cells.

The origin of the other set of fibrils, which always lie in and form the walls of all types of cells, is very obscure and discussion of them is reserved for another paper. These fibrils are always developed and in position before the cell wall is exposed to the air and chitinized thereby. Being of a different chemical composition these fibres are never chitinized but remain in position in the chitin matrix. Their terminals are always naked and exposed, no matter how heavy the chitin may be around them.

These fibril openings through the chitinous wall can be and are modified along with the fibril products in various functional ways. These modifications of the fibrillations of the epidermal cells form a most interesting chapter in the structure of insects. The writer holds that this structure is universal to the hypodermal cells of insects. In thousands of instances studied we have yet to find any type of such cells in which these fibrils do not perforate the chitin walls and establish connection with the outside world.

To observe these structures to best advantage the material must be well preserved and should undergo thorough staining,—preferably in Iron-alum-hæmatoxylin. The tissue should lie in the mordant two days and in the stain an equal time. This allows the stain to penetrate the fibrils through the chitin matrix which is very resistant to the stain.

The sensory cells of the epidermis may be modified from the type in various ways. They may be sunk below the surface as in Hicks' vesicles, or compounded into pore-plates beneath (or flush with) the surface, or raised into variously formed spines, scales, hairs, pegs, etc. No matter, however, what the form or elevation or degree of complexity the main structures are similar to those diagrammed in the typical cell (Fig. 2). In the later figures are given a few of the more characteristic variations from the typical epidermal cell.

Fig. 3, is a peg from the antenna of a wasp, *Vespa*. It has a membraneous tip through which the tip of a bundle of nerve fibrils

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are protruding. They extend quite a distance beyond the membrane and stain nicely.

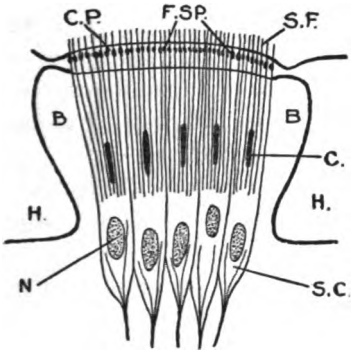

Fig. 4. Pore Plate, *Vespa* antenna. S.C. —sense cell; N.—nucleus; H.—chitin hypoderm; C.—centrosphere; B.—blood space; S.F.—sense fibrils; F.SP.—fibril spheres; C.P.—chitin pore-plate.

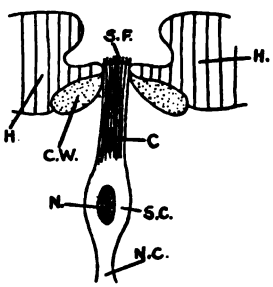

Fig. 5. Hick's Vesicle, Honey Bee. S.C. —sense cell; N.—nucleus; N.C.—nerve connection; C.—centrosphere; C.W.—chitin wall of cell; H.—hypoderm; S.F.—sense fibrils.

Fig. 4, is a diagrammatic view of a compound pore-plate from antenna of Vespa. This pore plate has a slit lengthwise of it in a surface view; many Ichneumon flies have this form with very enlongate slits. The pore plate is penetrated by the fibrils from the group of cells which compose it. The nuclear end of the cell may be extended by growth to a remote position from what it originally occupied.

The space (G) is filled with blood fluid and may by excitation and flow of blood to the antenna slightly protrude the pore plate which is suspended by a thin membranous ring. They might properly be termed erectile pore plates. At (E) on the diagram is a row of darker staining spheres which we will call for want of better name—fibril centrospheres. The ontogenetic origin of this body from the parent centrosphere and its subsequent functional history, have never been worked out, so far as we know. These pore plates, wherever found in insects and related animals, always have exposed fibrils extending through them. They are not *solid chitin disks* as assumed by many writers.

Fig. 5, is a diagram of Hicks' vesicles, which are a form of sunken peg with a membranous top through which the nerve fibrils

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are exposed. The chitin body (D) is the wall of the sunken peg cell.

Some writers consider this form of cell alone capable of receiving olfactory stimuli, denying that all homologous pegs which are raised varying degrees above the surface are capable of such function. The writer desires to stress the fact that these vesicles of Hicks' (1868) are but sunken pegs; and are not different in main details from those raised above the surface.

Fig. 6, is a diagram of the sense scale from the antenna of the crustacean, *Oniscus*. This shows that the nerve fibrils are exposed in a similar manner in one of the related families of the Arthropods. The writer has seen extensive areas around the mouth parts of spiders where these fibrils are exposed on flat membranes.

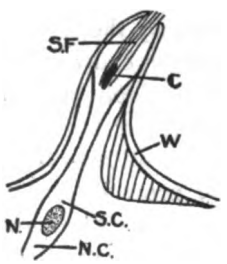

Fig. 6. Sense Scale, *Oniscus* antenna. S.C.—sense cell; N.—nucleus; N.C.—nerve connection; W.—wall of scale; C.—centrosphere; S.F.—sense fibrils at tip.

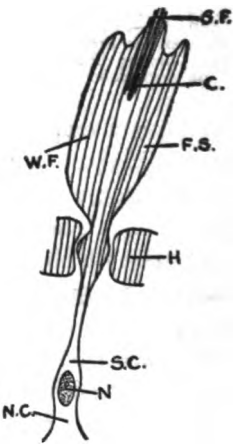

Fig. 7. Sense Scale, *Pyrameis* antenna. S.C.—sense cell; N.—nucleus; N.C.—nerve connections; H.—hypoderm; W.F.—wall fibrils; F.S.—flat part of scale; C.—centrosphere; S.F.—sense fibrils.

Fig. 7, is a diagram of a scale from the antenna of a butterfly—*Pyrameis*.

From observation of many thousands of these various scale types the writer is convinced that the evidence shows many forms of epidermal scales which have all the cytological details necessary to receive in some degree olfactory stimuli.

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As to what extent any particular type or set of cells actually do receive such stimuli we deem it impossible at present to state. Experiments which show that insects can smell both with and without the antennæ are of little value.

Some authors contend that the Hicks' vesicles on the legs are olfactory in function, while they absolutely deny that apparently homologous cells on the antennæ and palpi are capable of receiving such stimuli. The antennæ and palpi are but leg rudiments, a pair from each of the five segments of which the head is composed, being modified for various uses. In some forms of insects such as some Chironomidæ the antennæ are becoming rudimentary, while the first pair of true legs are greatly enlongated and used as sense organs, being extended toward and waved toward objective points. In other insects which do not feed in the adult state, such as some Ephermeridæ, the antennæ are very rudimentary.

Again some insects which do not feed in adult stages, such as some families of moths, have very large and much branched antennæ. In this case they seem to be connected with sexual functions. An almost endless list could thus be given of the modifications of the various forms of legs in the different insect families. These legs are homologous. There is nothing to indicate that their epidermal cells may not be homologous also. The minute structure of these cells as detailed above further reinforces the conviction of their homology. But the fact remains that all insects respond to odors, no matter what the modificactions of the epidermal appendages.

Many experiments have been devised by various students to try and show by mutilation which organs are olfactory and which not. Antennæ, palpi, tongues, legs, wings, styles, halters, stings, etc., have been removed in an endeavor to locate the olfactory sense. While the results are equivocal, most observers agree that the sense is not confined to any one set of appendages. We believe that the antennæ can and do act as receptors of olfactory stimuli under normal conditions, both for the reasons given above and because of observations upon the animals. Extensive observations were made this summer on normal insects engaged in feeding and other duties, to try and determine this point. For many days the

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various bees which visited a patch of columbine were observed. As the nectary tubes of these flowers are too long for the tongue of the bees, they invariably went to the rear of the flower and ripped the nectary open with the tongue. The bee working on the outside of the flower was in good position to show the normal use of the antennæ. The antennæ were invariably waved toward, then placed in direct contact with the nectary, and the presence or absence of food determined thereby. Out of thousands of bees which visited the flowers not one was observed to feed without first making an examination with the antennæ.

Many ants were studied in their travels, and the antennæ were found to be constantly in use. When near enough, making contact with the object; when further away, waving them towards the object.

Many species of Ichneumon flies were observed. Their antennæ are almost constantly in motion, apparently receiving most of their stimuli without contact.

For these reasons we believe that there are many types of epidermal cells widely scattered over the body which are normally capable of and do receive olfactory stimuli.

The arbitrary assumption of this function as belonging to any one type of cells without adequate cytological investigation can but lead to scientific mistakes. The call is for more and better cytological work.

Battle Creek, Mich. E. W. ROBERTS.

ARACHNOIDISCUS IN MARYLAND DIATOMACEOUS EARTH

About a year ago a friend in Maryland sent me a piece of diatomaceous earth which was uncovered by the excavation for a State road. It did not look very promising, being very sandy. Some time later, however, I cleaned a part of it and during the process was rather disgusted to find fragments of *Arachnoidiscus* mixed with it. It never seemed possible that the diatom might belong there, so I cast the stuff away and started anew only to find them in the new batch. I then became suspicious, but remembering an article I had read by Kitton in an old number of Science

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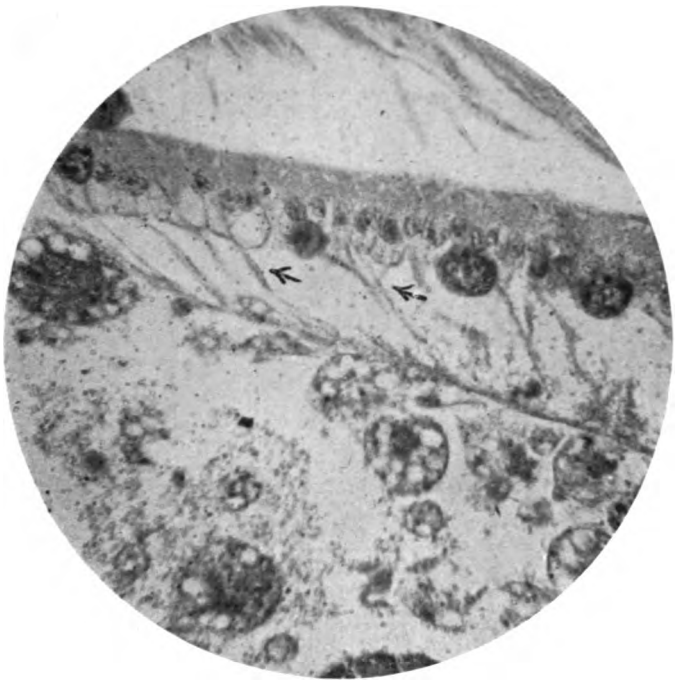

Fig. 1. Nerve Fibrils to Ectodermal Cells in Tussock Moth.

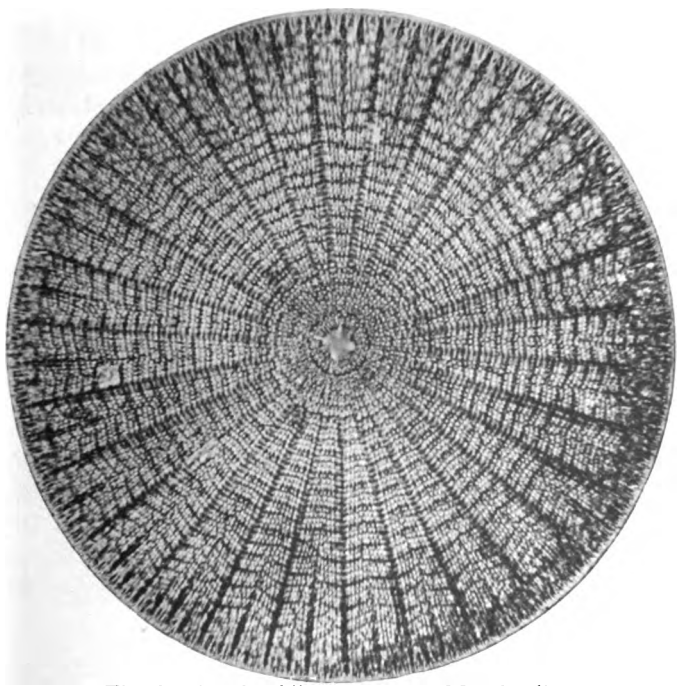

Fig. 2. Arachnoidiscus ornatus, Marylandica.

PLATE XVI

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Gossip in which he attributed the finding of a frustule of *Arachnoidiscus* in British waters to the use of dirty cleaning vessels, I secured an entirely new set of glasses and started again. The result was conclusive. *Arachnoidiscus,* apparently *ornatus,* was there, plentifully, together with *Actinoptychus calicinus,* a form I have never before seen in the Maryland deposit.

Repeated cleanings have verified the find. I have since visited the locality and made careful examination of the bank from which it came.

The Arachnoidiscus apparently occurs only in the topmost part of the deposit, and a few feet underneath the level at which it occurs most plentifully it disappears entirely when we reach the typical rich bed of diatomaceous earth. Another outcrop about five hundred yards away also shows it; but elsewhere I have been entirely unable to find it.

The deposit, a part of the Maryland Miocene, of which the Richmond beds form a part, is at a point known as Bird's Hill on the State Road about nine miles south of Annapolis. At this place is about one hundred feet above sea level and is the highest point I know of at which this earth shows. The deposit is only a few feet above sea level at Ferry Landing opposite Nottingham and at the Calvert Cliffs on the Chesapeake Bay, both these latter places being about twenty-five miles south of Bird's Hill.

The Arachnoidiscus here mentioned closely resembles *Arachnoidiscus ornatus* of the Pacific and I have taken the liberty of naming it *Arachnoidiscus ornatus,* variety *Marylandica.*

I may be mistaken in thinking I have really made a discovery and regret that the great authorities are no longer alive to pass on it but I can find nowhere any reference to such a find either in Atlantic waters or in the fossil deposits adjacent thereto.

I am sending with this mounted specimens which I hope may be reproduced for the benefit of your readers who may be interested. (See Pl. XVI, Fig. 2).

BLANTON C. WELSH,
U. S. Army, Retired.

Montclair, New Jersey,
July 8, 1915.

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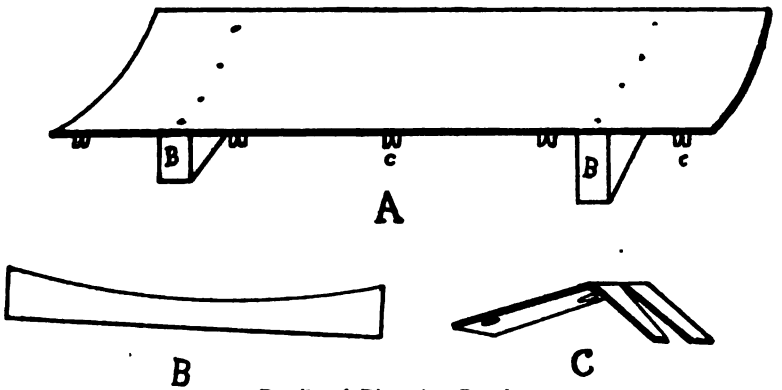
A SATISFACTORY DISSECTING BOARD

The dissecting of the cat, dogfish and some of the smaller animals caused in my laboratory, as I presume it has in others, a demand for an adequate dissecting board. In taking charge of a course in cat anatomy seven years ago there came into my possession also some dissecting boards supplied with plumb-bobs and



Details of Dissecting Board.

strings for spreading and holding the specimens in place. Such trays are little if any better than a plain board with nails driven in the sides to which the strings may be tied. Both of these types were replaced by the simple piece of apparatus herein described which has given complete satisfaction.

The principle part of the device consists of a board 70 cm. long, 40 cm. wide and 12 mm. thick screwed to two cross pieces B 30 mm. thick, one of which is 55 mm. high and the other 75 mm. high. These pieces are cut in such a way as to give a dish to the board 12 mm. deep. The difference in the heighth of the two pieces gives a sufficient slope to carry away any liquid that might come from the specimen. To either side of the board are fastened five iron cleats as shown in the figure (A). Each cleat C is bent at an angle of 120° and the free end, 15 mm. long, carries a V-shaped slot for its full length. The slot at its open end is 4 mm. wide The specimen is held in place and spread by means of pieces of Jack-chain (No. 16 or 18) 20 cm. long provided with fish-hooks

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with the barbs removed. After the hooks have been attached to the animal the chains are drawn into the slots of the cleats where they are held firmly.

University of Utah. NEWTON MILLER.

MECHANISM OF MENDELIAN HERIDITY

In a book bearing this title T. H. Morgan and co-workers have undertaken to bring to the general biologist the significant steps that have been made by the geneticists in the study of inheritance, and the correlation of these results with the discoveries made by the students of the minute structure of the germ cells. It is perfectly apparent that these cells, the egg and the sperm, must contain the mechanism by which hereditary qualities are handled from generation to generation. It is manifest that the wonderful results which the Mendelians have been able to get by breeding experiments will be explained, if ever, only through the study of the minute units of cytoptasmic and nuclear structure, which must be their material basis.

As is well known the author strongly supports the view that the chromosomes are the particular part of the protoplasm which determines such hereditary phenomena as follow the Mendelian principles. No cytological work has done more to discover the facts which tend to support this view than the brilliant investigations of Professors Wilson and Morgan and their students in Columbia University.

This book brings together the wonderful series of parallels between the observed conditions of the chromosomes and the statistical behavior of unit characters in breeding, which we describe collectively as Mendelian. For example, the observed segregation of chromosomes in the genesis of the germ cells coincides remarkably with the theoretical demands of the Mendelian principles of segregation resulting in the "purity of the gametes." Similarly there has been established in some organisms a parallelism between the number of chromosomes and the number of hereditary *groups* of characters; also in some degree between the size of the chromosomes and the size of these groups of characters.

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A more difficult task outlined and undertaken in this book is to show that there are parallels in the behavior of the chromosomes to those more complex dissolving of groups of characters which allows more or less freedom (and more or less linkage) of one unit quality to other unit qualities. If chromosomes carry the unit characters it is clear that each chromosome must carry factors relating to numerous unit characters, since there are many more characters than chromosomes. From the way that different unit characters behave from generation to generation, it is clear also that there must be machinery for the separation and crossing over of the factors of the unit characters from one chromosome to another in differing degrees. The authors find this possibility in the twisting, the partial unions, and the separation along new lines that occur in the period of conjugation of the chromosomes in the maturation of gametes.

Brief statements are made of other offered explanations of the Mendelian phenomena of inheritance.

The discussion is given under the following Chapter headings: Mendelian Segregation and the Chromosomes; Types of Mendelian Heredity; Linkage; Sex Inheritance; Chromosomes as Bearers of Hereditary Material; Correspondence between the Distribution of the Chromosomes and of the Genetic Factors; Multiple Allelomorphism; Multiple Factors; The Factorial Hypothesis. The Appendix contains directions for the breeding of *Drosophila*, the fruit fly on which much of the investigation of Mendelian phenomena in animals has been made; a Bibliography; and an index. Mechanically the book meets every expectation.

Mechanism of Mendelian Heredity, by Morgan, Sturtevant, Muller, and Bridges. 262 pages, 64 illustrations. Henry Holt & Co., New York, 1915. Price $3.00.

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CONSTITUTION

Article I

This Association shall be called the American Microscopical Society. Its object shall be the encouragement of microscopical research.

Article II

Any person interested in microscopical science may become a member of the Society upon written application and recommendation by two members and election by the Executive Committee. Honorary members may also be elected by the Society on nomination by the Executive Committee.

Article III

The officers of this Society shall consist of a President and two Vice-Presidents, who shall hold their office for one year, and shall be ineligible for re-election for two years after the expiration of their terms of office, together with a Secretary, a Treasurer, and a Custodian, who shall each be elected for three years, be eligible for re-election, and whose terms of office shall not be coterminous.

Article IV

The duties of the officers shall be the same as are usual in similar organizations; in addition to which it shall be the duty of the President to deliver an address during the meeting at which he presides; of the Custodian to receive and manage the property and permanent funds of the Society under the direction of the Executive Committee and in conjunction with a permanent committee to be called the Spenecr-Tolles Fund Committee, and to make a full and specific annual report of the condition of all the property, funds, and effects in his charge; and of the Secretary to edit and publish the *Transactions* of the Society.

Article V

There shall be an Executive Committee, consisting of the officers of the Society, three members elected by the Society, and the

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past Presidents of the Society and of the American Society of Microscopists who still retain membership in this Society.

ARTICLE VI

It shall be the duty of the Executive Committee to fix the time and place of meeting and manage the general affairs of the Society.

ARTICLE VII

The initiation fee shall be $3, and the dues shall be $2 annually, payable in advance. But any person duly elected may upon payment of $50 at one time, or in installments within the same year, become a life member entitled to all the privileges of membership, but exempt from further dues and fees. All life membership fees shall become part of the Spencer-Tolles Fund, but during the life of such member his dues shall be paid out of the income of said fund. A list of all life members and of all persons or bodies who have made donations to the Spencer-Tolles Fund in sums of $50 or over, shall be printed in every issue of the *Transactions*. The income of said fund shall be used exclusively for the encouragement and support of original investigations within the scope and purpose of this Society. The principal of the fund shall be kept inviolate; "Provided, however, that nothing in this constitution shall prevent the Executive Committee at any regular meeting from transferring the Spencer-Tolles Fund to a University, or other incorporated institution for original research, under such conditions as shall safeguard the permanence of the Fund, and its application to the general purpose for which it was intended; such power to be vested in the Executive Committee only after securing, and in obedience to, the expressed will of a majority of the constitutional members of the American Microscopical Society, or after the constitutional failure of said society.

ARTICLE VIII

The election of officers shall be by ballot.

ARTICLE IX

Amendments to the Constitution may be made by a two-thirds vote of all members present at any annual meeting, after having been proposed at the preceding annual meeting.

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BY-LAWS

ARTICLE I

The Executive Committee shall, before the close of the annual meeting for which they are elected, examine the papers presented and decide upon their publication or otherwise dispose of them.

All papers accepted for publication must be completed by the authors and placed in the hands of the Secretary by October 1st succeeding the meeting.

ARTICLE II

The Secretary shall edit and publish the papers accepted, with the necessary illustrations.

ARTICLE III

The number of copies of *Transactions* of any meeting shall be decided at that meeting. But if not decided, the Secretary shall, unless otherwise ordered by the Executive Committee, print the same number as for the preceding year.

ARTICLE IV

No applicant shall be considered a member until he has paid his dues. Any member failing to pay his dues for two consecutive years, and after two written notifications from the Treasurer, shall be dropped from the roll, with the privilege of reinstatement at any time on payment of all arrears. The *Transactions* shall not be sent to any member whose dues are unpaid.

ARTICLE V

The election of officers shall be held on the morning of the last day of the annual meeting. Their terms of office shall commence at the close of the meeting at which they are elected, and shall continue until their successors are elected and qualified.

ARTICLE VI

Candidates for office shall be nominated by a committee of five members of the Society. This committee shall be elected by a

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plurality vote, by ballot, after free nomination, on the second day of the annual meeting.

Article VII

All motions or resolutions relating to the business of the Society shall be referred for consideration to the Executive Committee before discussion and final action by the Society.

Article VIII

Members of this Society shall have the privilege of enrolling members of their families (except men over twenty-one years of age) for any meeting upon payment of one-half the annual subscription ($1).

Article IX

There shall be a standing committee known as the Spencer-Tolles Fund Committee to take general charge of the fund and to recommend annually what part of the income shall be expended for the encouragement of research, but the apportionment of the sum thus set apart shall be made by the Executive Committee.

The Spencer-Tolles Fund Committee shall also have general charge of the expenditure of such money as may be apportioned, under the conditions laid down by the Society for its use.

The Custodian shall be an *ex-officio* member of this committee.

Article X

The Executive Committee shall have the power annually to appoint two members to represent the Society on the Council of the American Association for the Advancement of Science, in accordance with the regulations of the latter organization.

Revised by the Society, December, 1913.

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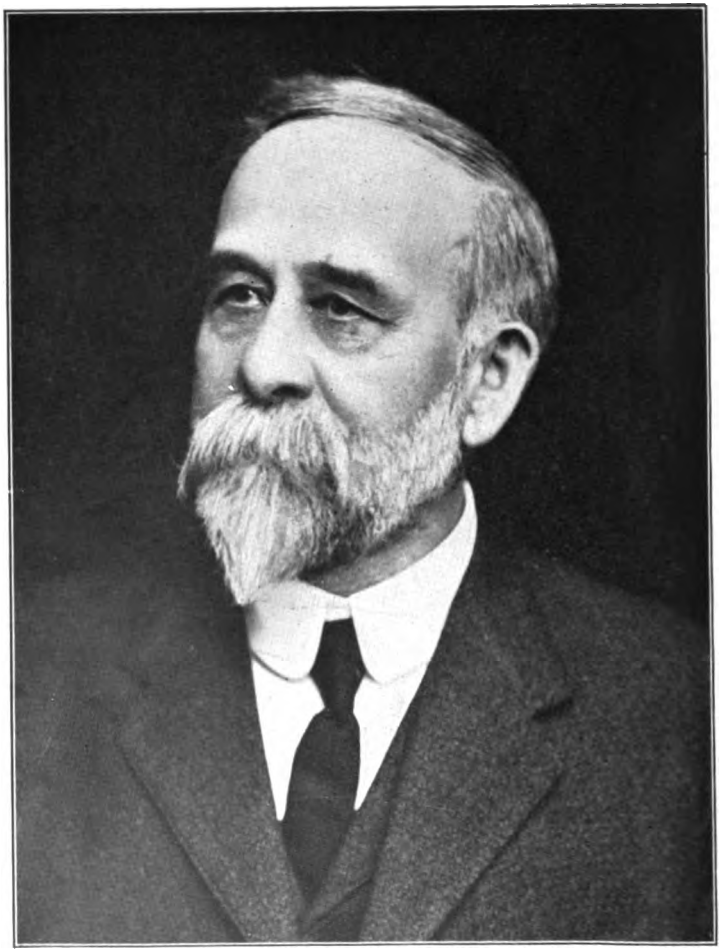

CHARLES EDWIN BESSEY

PLATE XVII

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NECROLOGY

BESSEY, CHARLES EDWIN, Ph.D., L.L.D., '98.....Lincoln, Nebr.
BODINE, DONALDSON, '96.....Crawfordsville, Ind.
GAGE, MRS. SUSANNE PHELPS, '87.....Ithaca, N. Y.
HYATT, JONATHAN DEUELL (Hon. Member)..New Rochelle, N. Y.
OHLER, W. H., '91.....Portland, Me.
TYRRELL, E. G. HARCOURT, '13.....Eshowe, Zululand, S. Africa

A SKETCH OF THE LIFE OF CHARLES EDWIN BESSEY

BY RAYMOND J. POOL

Charles Edwin Bessey was born in a log house on a Wayne County, Ohio, farm May 21, 1845. His parents who were of French and German descent were Adnah Bessey and Margaret Ellenberger Bessey. The earlier ancestors emigrated from the Continent to England during the period of extreme religious persecution which characterized the Huguenot days. Later some of them came to America and settled first in Pennsylvania and later migrated to Ohio about 1832.

Professor Bessey's education was received in the country schools and academies of Ohio. The preparatory days were frequently interrupted by periods of teaching, by the uncertainties accompanying the war and by the death of his father in 1863. But after several years of preparatory study in the lower schools Bessey entered the freshman class at the Michigan Agricultural College in July 1866. He completed the college course and was given the degree of bachelor of science on November 10, 1869 at the age of twenty-four.

When he went to the college Bessey was convinced that he wanted to become a civil engineer or surveyor since he had a great liking for the out-of-doors and had had some practical experience in surveying and a natural inclination for that line of work from his father. His father was a surveyor of note in northern

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Ohio. But young Bessey found that his mind was drifting toward things botanical. This tendency was discovered by some of his instructors who encouraged him to enter the field of botany. At first he spurned that encouragement and advice but as his college work drew to a close the inclination toward botany became more and more pronounced until finally he determined to become a botanist. This decision was plainly shown when he accepted an assistantship in horticulture and was placed in charge of the greenhouse at his Alma Mater immediately after his graduation. However that position was held for a very short time because in December of that year (1869) he accepted an offer to become instructor in botany and horticulture in the Iowa State College at Ames. He began what was to be a fifteen-year service in Iowa in February, 1870.

In 1872 he was promoted to a full professorship at Ames and during the same year the Michigan Agricultural College conferred the degree of master of science upon him. Still later he was made professor of botany and zoology at Ames and in 1874 he went to the University of California by invitation of the president to deliver a series of lectures on botany. As one examines the outlines of the lectures which were delivered in that course it is noted that they were dominated by systematic botany of that day but they also contained an excellent summary of the whole field of botany as it was then known. The sequence in the taxonomic part of the lectures was from lower to higher forms but what is most significant is that with the flowering plants the sequence followed was the Benthamian *reversed*.

At Ames Bessey soon became the president's helper and later his confidential friend. The president found that his services were of much value in his office and upon the grounds of the college. Many of the fine trees and walks and drives that now grace the campus at Ames College were planted or laid out by Bessey or under his supervision. Because of this close association with the president Bessey first secured a near view of the methods and management of higher educational institutions. As he once remarked: "This work was all outside my regular duties and I received no extra money for it but it paid in what it did for me." How true

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that was can be best appreciated by those who were so fortunate as to be closely associated with him in his later years when all of the values of the earlier experiences were crystallized into a most fruitful and inspiring educational climax.

An incident of great importance to Professor Bessey was his first meeting with Dr. Asa Gray which occurred at the Dubuque, Iowa sessions of the American Association for the Advancement of Science in August 1872. Gray was the retiring president of the Association at that time and Bessey was elected to membership during those meetings. Professor Bessey also met many other men who were to become his long-time friends and co-laborers in botany and in the American Association at that meeting. His initial meeting with Dr. Gray was especially pleasant and resulted in arrangements being made whereby Bessey went to Harvard during the winter of 1872-1873 where he studied under the great master botanist. The subject of botany had been so richly illumined by Dr. Gray during those three months that the young western botanist returned for another period of work at Harvard in the winter of 1875-1876. Those months of enthusiastic study with Gray and the years of extremely pleasant correspondence that followed were among the most delightful memories of Professor Bessey's later years.

Bessey had served the state of Iowa in a varied educational and scientific program for a period of fifteen years when in June, 1884 he received notice that he had been elected to a professorship in the University of Nebraska. The election had been made without his knowledge but he went to Lincoln "to look the place over." He found that there was no laboratory for botany and that there was no botanical equipment whatever at Nebraska so naturally he was loath to leave his well established department at Ames. The position was accordingly refused. However a second offer was made to include the deanship of the industrial faculty and in that form Bessey accepted the call. The inaugural address was delivered in September 1884 but Professor Bessey did not move to Lincoln until after the completion of the college year at Ames, November 18, 1884. He began his first class work at the University of Nebraska in January 1885.

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The moment that Bessey arrived in Lincoln he became an enthusiastic citizen of his new city and state and he began at once to make himself at home in the new position. In doing that he was but true to the request of his mother expressed many years before when Bessey as a mere boy was leaving his home in Ohio to go to certain work in Michigan. Upon that occasion his mother said to him: "Charles, wherever you are be one of the people of that community; act as though you intended to live there always, and make yourself so useful that they cannot spare you." The degree to which he fulfilled that request when he went to Michigan, to Iowa and finally to Nebraska must have been one of the most gratifying recollections of his aged mother as she reviewed the career of her boy especially at the times of his visits to the old Ohio home which were made annually until the mother passed away at a ripe old age only a few years before the death of her illustrious son.

The state of Nebraska loved Dr. Bessey and during his life of more than thirty years in this state he reciprocated that affection to the fullest. But that was only one of the many directions toward which an overflowing measure of devotion and enthusiasm carried that good man. His broad-mindedness coupled with the many-sidedness of his personality made Bessey a most valuable citizen of the state. His intellectual horizon was wide enough and his sympathies deep enough to include the great and small affairs of the state and its people and to stimulate the development of the highest and best attainment in all of those with whom he came in contact.

No one was more active in the scientific organizations of the country than Bessey. From that first meeting of the American Association at Dubuque he seldom missed one of the annual gatherings of the numerous societies to which he belonged. He was always a conspicuous figure at such meetings where his active cooperation and his wise counsel were often sought and as often freely given. As the years wore on and his associates and former students became more and more numerous and widely scattered there were times of most delightful reunions especially between the master and his pupils.

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Many scientific honors were conferred upon him during his long association with American men of science. In 1880 he was made a fellow in the American Association for the Advancement of Science. He was president of the Society for the Promotion of Agricultural Science and of the Western Society of Naturalists in 1889. Upon four different occasions he was vice-president of the American Association and chairman of the botanical section. He was also a charter member of the Botanical Society of America of which he was president in 1895. From 1880 to 1879 he was botanical editor of the American Naturalist and from the latter date until the time of his death he held a similar position with Science. During this long period scarcely a paper or book appeared and escaped a review or at least a notice from his pen. As I have looked over collections of hundreds of such reviews I have been impressed with the tremendous amount of labor which all that necessitated. If one had in his possession all of the original books, papers, periodicals, bulletins, monographs, theses, etc., etc. which Professor Bessey reviewed or noted during that long service to science he would be equipped with a most excellent and fairly complete library of the more modern works on botany. That was a service which is probably not appreciated by one unfamiliar with its magnitude and significance. Perhaps the highest scientific honor which came to him was his election to the presidency of the American Association for the Advancement of Science at the Minneapolis meeting in 1910-1911. Dr. Bessey also enjoyed membership in many other scientific clubs, societies and academies in America and abroad. He secured the degree of Doctor of Philosophy from the University of Iowa in 1879 and the Doctor of Laws degree was conferred upon him in 1898 by Grinnell (Iowa) College.

Profesor Bessey was an effective speaker and a voluminous writer. Many of his earlier addresses and publications had a direct bearing upon the practical application of scientific botany to the every day life upon the farm and in the orchard, garden or forest or upon the wide grassy prairies and plains. Scores of articles of this sort were published in the farm papers of Iowa and Nebraska during his forty-five years' of labor in those two states. The well known series of textbooks which he began before he en-

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tered upon his professorship at Nebraska and the numerous technical papers are too familiar to the members of this society to require enumeration at this time. For more than a quarter of a century he worked upon the "phyletic idea" in taxonomy and the phylogenetic development of the various groups of the plant world. Botanists probably know of this phase of his contributions (after his texts) better than of any others. His last paper was entitled "The Phylogenetic Taxonomy of the Flowering Plants."

Of the many services Bessey rendered to science in general and to botany in particular which are of interest to the members of the American Microscopical Society perhaps the most interesting was the introduction of the laboratory method of instruction with the use of compound microscopes at Iowa State College in 1873. Possibly there were one or two other laboratories of that kind in America at that time but Bessey did not know of their existence. Later (1881) he gave the first botanical instruction in the University of Minnesota in which microscopic methods were included with the rest of the laboratory work. The microscopes for that course were borrowed from the college at Ames, since the University of Minnesota owned no such instruments at that time. Bessey also introduced that kind of instruction into the Nebraska schools after he had begun similar work at the University. He also published several papers dealing with microscopic plants and their relationships in the Transactions of the American Microscopical Society. Among the more important of those contributions may be mentioned those upon the phylogeny and classification of diatoms, desmids and other algæ and also upon bacteria and phycomycetous fungi. He was president of this society in 1902 and presided at the Pittsburg meeting in June of that year.

But if everything possible had been said of his numerous and varied contributions to science and of the many delightful and enviable features of his life among men we must still insist that we had not touched upon the most powerful and ever-broadening effects of this man's work because these were recorded in the classroom, in the laboratory, and all about the college and university as a captivating teacher and fatherly guide for the young. Possibly there have been greater botanists but there has never been a greater

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teacher of botany than Dr. Bessey. Few men ever enjoyed a broader knowledge and a wider point of view of his subject. He was at home in nearly every phase of the great study of plants. And he insisted that his students secure the very broadest training possible in the subject. The stimulating methods of the man and the *esprit de corps* that were constant delights about his department attracted hundreds of students from surrounding states and even from distant lands. The laboratories increased in number, the library and the herbarium grew in size and efficiency but never did this accretion catch up with the demands of his department. The herbarium of about 250,000 specimens was finally crowded out of the building. He was compelled to take the large classes to some other part of the campus and even there to divide them into smaller sections because there was not a room large enough to accomodate the whole class at one time. He was always crowded for room and it is peculiarly sad that he did not live to enjoy more commodious quarters in the new biological laboratory which is now in process of construction and which will bear his name. Dr. Bessey regarded teaching as his greatest work, as his greatest service to mankind and in that he labored with a joy and a bouyancy which would have kept him young for many additional years had not the long strain of the scarcely varied school room duties worn down the physical body and hastened the approach of the final crisis.

Charles E. Bessey was married to Miss Lucy Athern of West Tisbury, Martha's Vineyard, Massachusetts on December 25, 1873. To this union three sons were born, Edward, Ernst and Carl. The three boys graduated from the University of Nebraska with high honors. Edward and Carl specialized in engineering. The former died on July 12, 1910 while he was assistant professor of electrical engineering in the Colorado Agricultural College. Carl is at present assistant chief of a large engineering firm with offices in Chicago. Ernst followed his father's tendencies and specialized in botany. He is at present professor of botany in the Michigan Agricultural College where his father received his undergraduate training. Mrs. Bessey, the mother, retains her home at 1507 R Street Lincoln.

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JONATHAN DEUELL HYATT

Jonathan D. Hyatt, an Honorary member of the American Microscopical Society, died at New Rochelle, N. Y. Dec. 18, 1912, in his 88th year. Mr. Hyatt was a charter member of this society at its formation in 1878, and was elected to Honorary membership in 1905. He was its President in 1881.

He began his career as a teacher in the county schools of Dutchess County, N. Y.. After serving as principal in various high schools in New York State he became principal of the High School in the Borough of Bronx. He retired from teaching in 1904.

Mr. Hyatt was much interested in the natural sciences, and was a diligent worker with the microscope. He was one of the original members and a President of the New York Microscopical Society, a member of the Torrey Botanical Club, and Fellow of the Royal Microscopical Society. His anatomical studies of the honey bee and the cicada are among his best work in natural history.

His family is notably long-lived. His mother was 100 years and 6½ months old at her death; two brothers each lived to be 86½ years of age; he is survived by a sister now 92 years old.

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JONATHAN DEUELL HYATT

PLATE XVIII

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VOLUME XXXV No. 1

George E. Coghill,
Department of Anatomy,
University of Kansas.

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OF THE

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VOLUME XXXV

1916

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MASONRY BASES FOR THE INSTALLATION OF MICROSCOPES AND THEIR ACCESSORIES, INCLUDING THE CAMERA LUCIDA AND THE MICROSCOPE CAMERA

By N. A. Cobb

MASONRY AS A BASE

It has long been customary in the best laboratories to mount instruments of precision upon heavy pillars having foundations located in wells in the ground and passing upward through the floors without contact, the object being to prevent the tremors of the building from being transmitted to the instruments. The earth receives and nullifies the tremors.

The microscope has not often* received such special attention, notwithstanding the fact that whenever high powers are used, and especially when photomicrographs or high power camera lucida drawings are being prepared, vibration is objectionable. For many years I have had microscopes mounted on solid bases and strongly favor this method of support.

THE USE OF GIRDERS

One such installation was carried out in cement and steel as shown in Fig. 1.** Three girders, two approximately eight inches in each transverse dimension, and between them a third smaller one,

*The author knows of only about twenty of the massive installations such as are described in the following pages.

**The illustrations were made under the personal supervision of the author by Mr. E. M. Grosse and Mr. W. E. Chambers. They show so much detail, so nearly to scale, that any good mechanic can build from them.

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are imbedded vertically to the depth of several feet in a block of cement weighing many tons located under the building. The middle short girder, extending 18 inches above the floor, carries the microscope and certain accessories connected with illumination. The two tall, paired girders extend to within eighteen inches of the ceiling, projecting upward into the room about eleven feet. The wooden floor was laid tightly about the girders after they had been set in the cement and everything was then given a few months in which to settle into permanent position, after which an ordinary key-hole saw was run through the floor entirely around the contour of each girder, so that each cleared the flooring and floor-covering by the thickness of a saw blade. See 3, 43, etc., Fig. 1.

The girders, which clear the wall of the room by an inch or two, carry the accessory apparatus, which is attached to them in some instances by means of sliding one-sixteenth-inch sheet-metal sleeves that may be clamped at any desired height, in other instances by other means. All parts are dead black. The sleeve of the small central pillar projects outward at the top, that is, toward the observer, so as to form a microscope shelf one to two times larger than the base of the microscope. The sleeve carrying the microscope is clamped to its pillar by set-screws, and can be set high or low to suit different operators and different classes of work. When photographing it is better to set the microscope near the floor, so as to bring the camera (19, Fig. 1) low enough to make it unnecessary to use a step-ladder in focusing. By placing the microscope high and the drawing table low, one can obtain for camera lucida work a distance of two and one-half feet between the level of the eye-piece of the microscope and that of the drawing table.

The sleeve carrying the microscope carries also a large vertical wooden front, two feet wide and as long as the microscope window is wide. This wooden front or screen slides up and down with the microscope, and has in it two apertures, one in front of the microscope mirror, designed to allow light from the sky, or from an illuminating screen, to strike the mirror and pass thence through the microscope; and the second to secure a correct illumination of the drawing board when the camera lucida is in use. See Fig. 1. This latter aperture is much the larger, is glazed with ground

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glass, and is opened and closed at will by means of a light, suspended, opaque slide worked up and down by foot-power.

The microscope window faces the sun, and is fitted with two light-proof roller blinds, one just behind the other, so that the sun's light may be shut off or allowed full access. The roller blinds move in lateral grooves ten inches deep, a depth sufficient to prevent the blinds from troubling by bellying on windy days. As a further provision against wind action, tight wires may be strung horizontally on the window frame inside the blinds. The blinds may be of any opaque material, and if long, should be thin. Ordinary opaque window blinds can be sized black so as to become practically light-proof, and since it is advisable to have two blinds, such sizing wholly excludes the light. Ordinary spring rollers are used and are boxed in at the top in a light-tight manner.

CARRIERS FOR THE ACCESSORIES

The right-hand lower sleeve carries a leg-of-mutton shaped shelf or table for use in making camera lucida drawings. The sleeve, like all the other supports is balanced with a sash-weight, so as to move freely up or down through a range of several feet. The shape of the shelf has been evolved from years of use, and gives the investigator free play for hands and body. See 33, 34, Fig. 1.

At the left is a similar sleeve and shelf used for a different purpose,—although, as it is the mate to the camera lucida table, it could, in the case of a left-handed operator, be used as the right-hand table would be used by a right-handed operator. The usual position for the left-hand shelf is, however, about on a level with the microscope stage; first, because that is about ordinary table height and is convenient for supporting the dissecting microscope, which has a special illumination of its own (See Fig. 1); and second, because preparations may then be moved on and off the stage of the microscope with the least danger and with the greatest facility; a third reason is that in this position the left forearm finds it a most convenient rest in working the fine adjustment screw of the microscope, as shown in Fig. 3.

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The two long girders also carry two strong vertically adjustable cross-pieces for the attachment of accessories above the microscope. Set-screws are provided for clamping the cross-pieces. Arms extend upward from the cross-pieces to carry anti-friction pulleys travelling on edges of the pillar. The microscope camera (See 19, Fig. 1,) hangs above the microscope in readiness for instant use, and is of vertical pattern, carrying the photographic plate in a horizontal position. In focusing, the cross-piece carrying the camera is moved upward or downward; a scale on the pillars gives the various magnifications. The operator loosens two hooks and the camera front drops instantly into position on the end of the microscope barrel. The whole is ready for use in a few seconds' time. If the exposure is long, one leaves the instrument during the exposure with the greatest confidence that nothing can disturb it; tremors in the building will not be received either by the microscope or the photographic plate.

CAMERA LUCIDA

A second attachment of prime importance in producing illustrations is the peculiar camera lucida. Fig. 1, 17, 24.

The history of the camera lucida is a very interesting one. It is impossible to go into details here, but nothing is clearer than that this instrument is one of great importance to the microscopist, and its history is in accordance with this fact. The utmost ingenuity has been exercised to produce an instrument by means of which sketches of small objects can be made with the aid of the microscope. The necessity for this class of work is very great. The photographic camera is inadequate for most objects. Only in the case of smears or exceedingly thin sections, or natural objects of great thinness, is a photomicrograph satisfactory. In all other cases, in order fully to elucidate the structure by means of an illustration, it is necessary to represent the appearances at different depths in the preparation. This can be done only by focusing the microscope for each particular depth. This fact, thus hastily explained, is what makes it absolutely necessary to use a camera lucida for the proper representation of most microscopic objects. This fundamental necessity is what has given rise to the many

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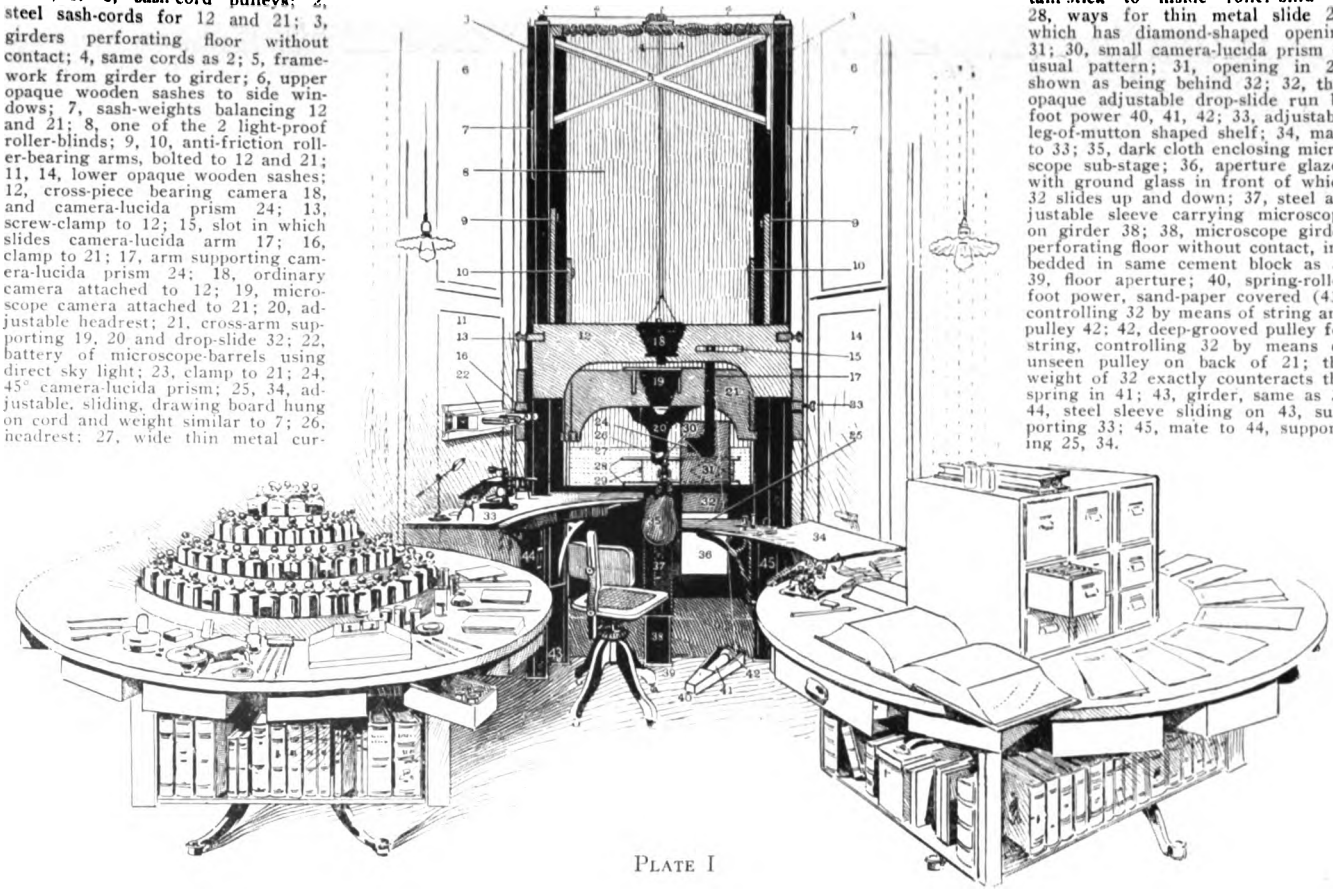

FIG. 1.—1, sash-cord pulleys; 2, steel sash-cords for 12 and 21; 3, girders perforating floor without contact; 4, same cords as 2; 5, framework from girder to girder; 6, upper opaque wooden sashes to side windows; 7, sash-weights balancing 12 and 21; 8, one of the 2 light-proof roller-blinds; 9, 10, anti-friction roller-bearing arms, bolted to 12 and 21; 11, 14, lower opaque wooden sashes; 12, cross-piece bearing camera 18, and camera-lucida prism 24; 13, screw-clamp to 12; 15, slot in which slides camera-lucida arm 17; 16, clamp to 21; 17, arm supporting camera-lucida prism 24; 18, ordinary camera attached to 12; 19, microscope camera attached to 21; 20, adjustable headrest; 21, cross-arm supporting 19, 20 and drop-slide 32; 22, battery of microscope-barrels using direct sky light; 23, clamp to 21; 24, 45° camera-lucida prism; 25, 34, adjustable, sliding, drawing board hung on cord and weight similar to 7; 26, headrest; 27, wide thin metal curtain-stick to inside roller-bind 8; 28, ways for thin metal slide 29, which has diamond-shaped opening 31; 30, small camera-lucida prism of usual pattern; 31, opening in 29, shown as being behind 32; 32, thin opaque adjustable drop-slide run by foot power 40, 41, 42; 33, adjustable leg-of-mutton shaped shelf; 34, mate to 33; 35, dark cloth enclosing microscope sub-stage; 36, aperture glazed with ground glass in front of which 32 slides up and down; 37, steel adjustable sleeve carrying microscope on girder 38; 38, microscope girder perforating floor without contact, imbedded in same cement block as 3; 39, floor aperture; 40, spring-roller foot power, sand-paper covered (41) controlling 32 by means of string and pulley 42; 42, deep-grooved pulley for string, controlling 32 by means of unseen pulley on back of 21; the weight of 32 exactly counteracts the spring in 41; 43, girder, same as 3; 44, steel sleeve sliding on 43, supporting 33; 45, mate to 44, supporting 25, 34.

PLATE I

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PLATE II

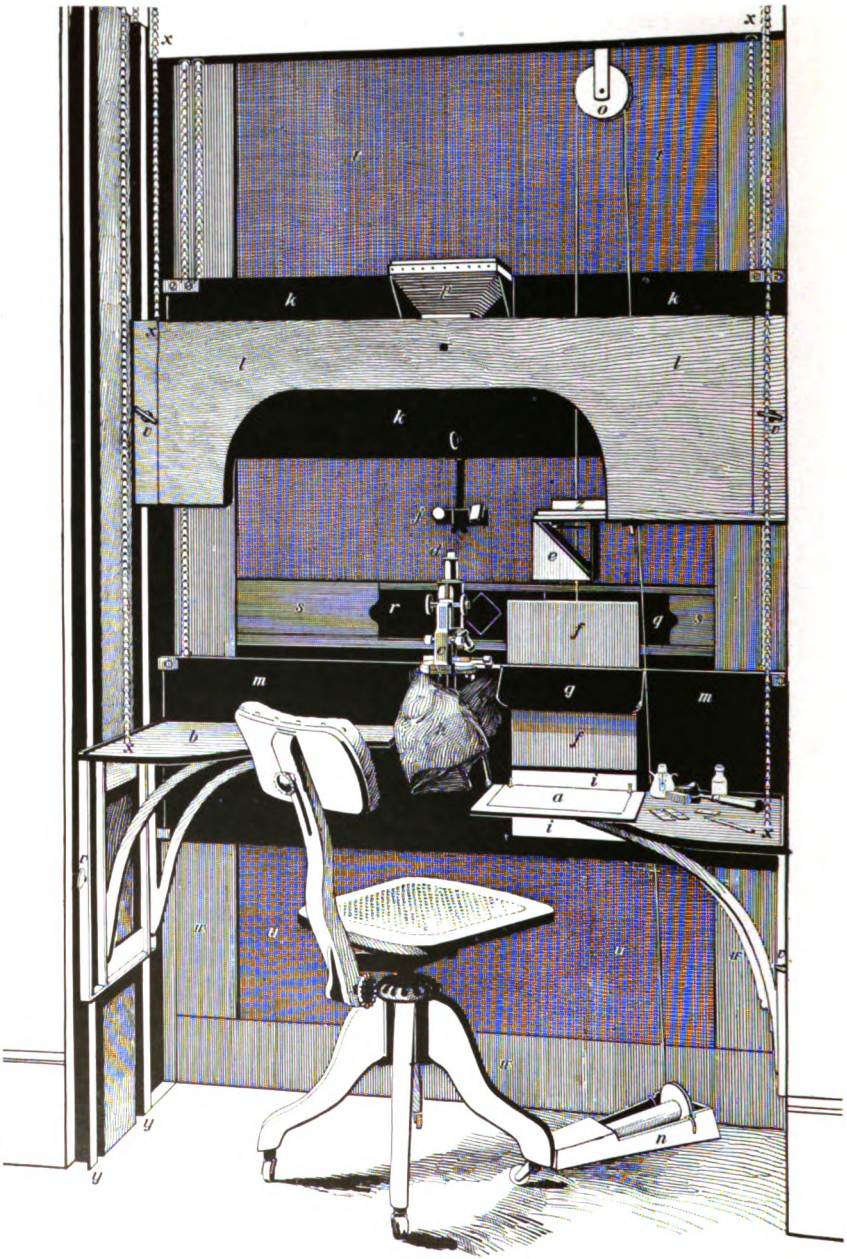

FIG. 2

a, drawing board pinned to right-hand shelf; *b*, left-hand shelf; *c*, microscope; *d*, small camera-lucida prism; *e*, large camera-lucida prism; *f*, opaque slide, moving in front of the aperture, *i*, by means of the foot power, *n*; *g*, hinged flap to prevent direct light from reaching the eye of the operator; *h*, cloth surrounding the base and sub-stage of microscope; *i*, aperture in the cross-piece *m*, glazed with ground glass; *j*, adjustable headrest; *k*, cross-piece carrying the micro-camera *p*; *l*, cross-piece supporting prism *e*; *m*, cross-piece supporting the microscope; *n*, foot power which, by means of a string pass-

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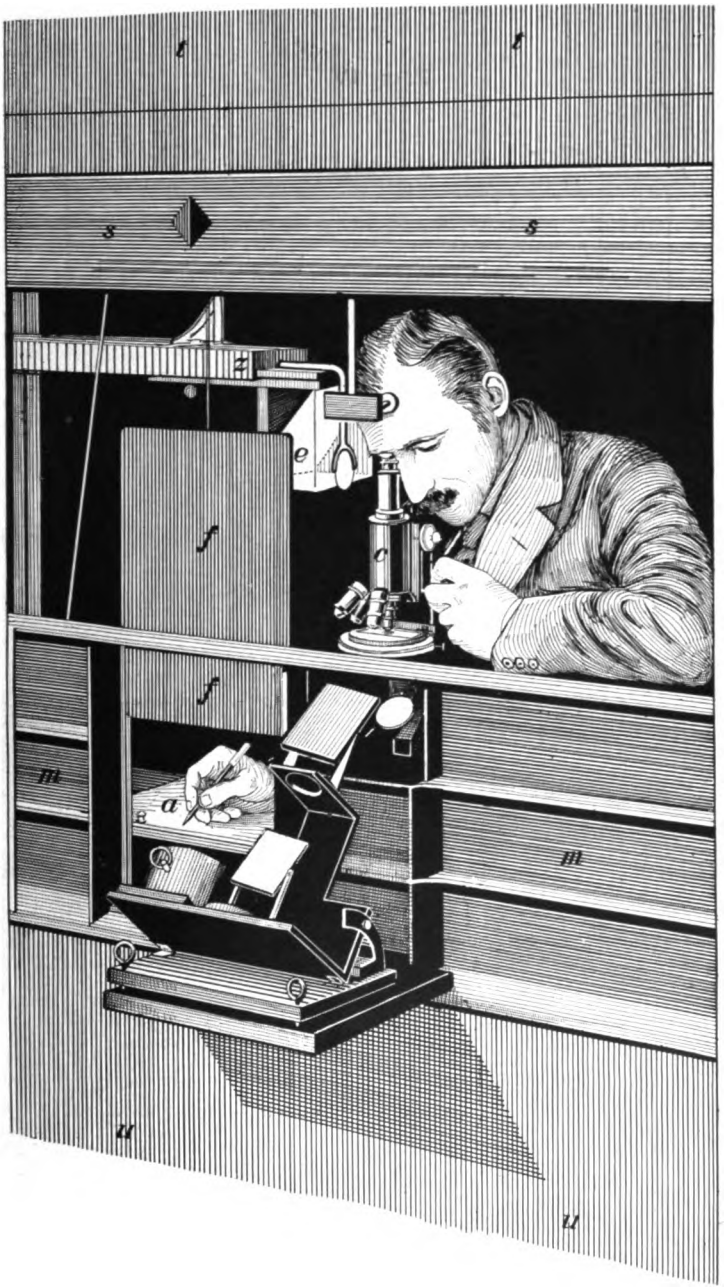

FIG. 3

ing over the pulley *o*, works the slide *f*, *o*, pulley; *p*, micro-camera; *q*, *r*, thin opaque slides with diamond-shaped openings sliding in front of a glazed aperture in the wide, thin curtain stick *s*; *t*, opaque blind rolling upward carrying with it *q*, *r*, *s*; *u*, roller blind rolling downward carrying with it *g*, *h*, *m*; *v*, set-screws for the shelves *a* and *b*, as well as for the cross-pieces above; *w*, ten-inch grooves forming light traps for the blinds *t* and *u*; *x*, chains passing over pulleys to sash weights by which the various sliding parts are counterbalanced; *y*, z-shaped girders bolted to masonry of the building.

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PLATE IV

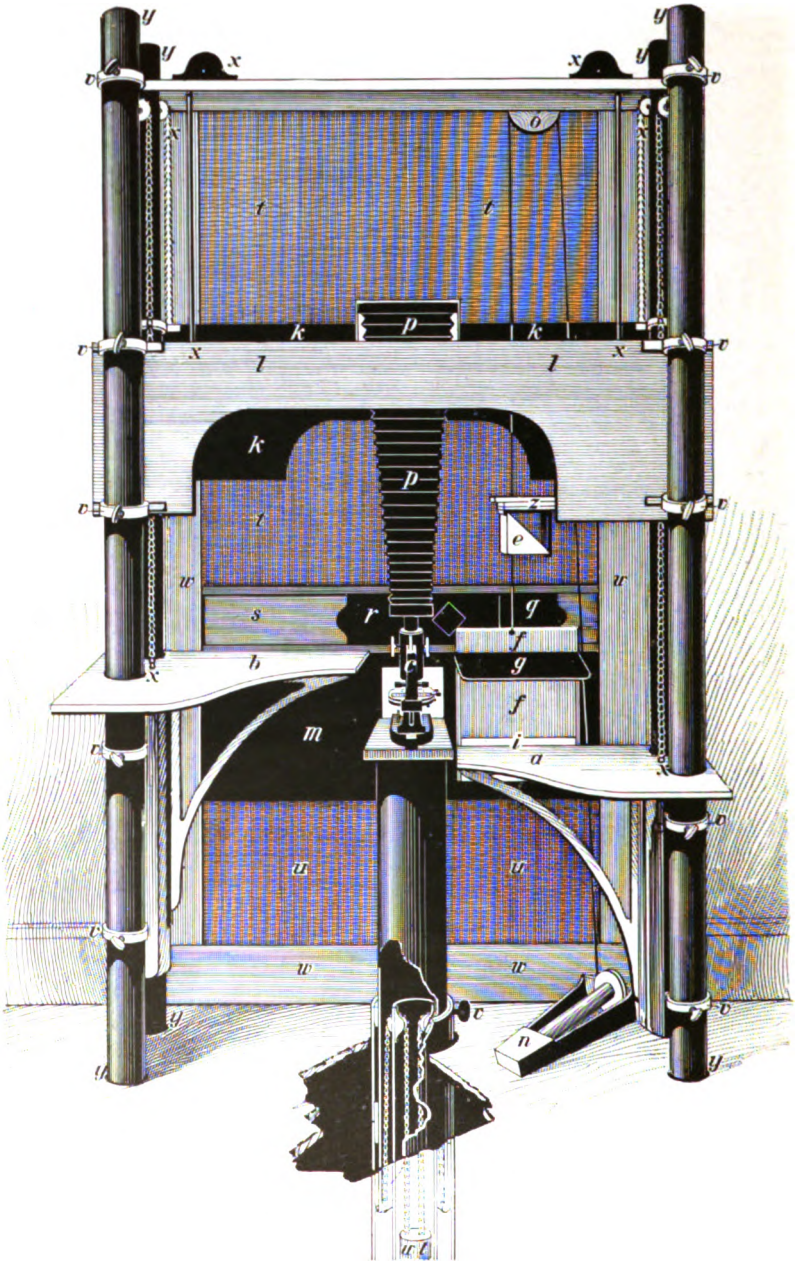

FIG. 4. Microscope installation carried out in iron tubing. Lettering approximately as in FIGS. 2 and 3

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patterns which the camera lucida has taken on during its development. The first instrument was an extremely simple one. From time to time improvements and additions have been made until at the present time the instruments issued by the best makers are marvels of ingenuity and workmanship. In fact, in the writer's opinion, they are almost too ingenious, for it appears to him that the various additions instrument-makers have made to the camera lucida during recent years, while they do accomplish the object aimed at, do so in an unsatisfactory manner.

To produce a good camera lucida drawing, it is necessary to have such light passing through the microscope as will enable the operator to see the object with the greatest possible clearness; and it is equally necessary so to control the light from the drawing board as to enable him to see his pencil-point at all times with the greatest possible clearness. With most objects it is impossible to secure this adjustment once for all, and for all portions of the drawing. Different portions of the object transmit or reflect different amounts of light, and, as this light varies it is necessary, in order that the drawing may be made with the greatest ease and precision, that the light from the drawing board should be modified accordingly. This end has been sought in a variety of ways, and more than any other one thing the effort to achieve this end has added to the complexity of the modern camera lucida. When the modern instrument is in good order, it may, it must be admitted, in a way accomplish its object; the difficulty is that it is complex and easily thrown out of adjustment, and some of its parts easily become soiled and dusty so as to be a hindrance rather than a help. Again, no very suitable device has yet been furnished by manufacturers for modifying the light from the drawing paper, except by a series of steps. It is usual to interpose tinted glasses, until the right balance of light has been secured, but it often happens that the most desirable shade cannot be secured, and in any case by this method there is always being inserted between the object and the eye, or the pencil and the eye, or between both and the eye, various pieces of apparatus that must be regarded as necessary evils, objectionable from a number of points of view.

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A second defect presented by many camera lucidas is the "double" reflection due to a silvered glass mirror. This defect can be tolerated for a short time, but after several hours the double reflection of the pencil point becomes very tiresome to the eye.

Any form of camera lucida hitherto introduced is trying to the eye-sight. During many years of experience the writer has endeavored to reduce the risk of injury to eye-sight due to use of the camera lucida, and the following suggestions, embodied in the outfits here described, are the result of his experiments. First, he has substituted for the ordinary mirror a large, and therefore necessarily heavy, 45 degree glass prism mounted on a support separate from the microscope. (24, Fig. 1 and e Figs. 2, 3 and 4). The advantages of this substitution are: 1. As the prism is mounted separately, it may be of any desired size, and may be placed at a considerable distance from the eye-piece of the microscope, thus increasing the magnification of the drawing; the advisability of this increased magnification will be dwelt upon later. 2. There are no double or multiple images and less light is lost by reflection. The light passes from the drawing-point through the lower, i. e. horizontal face of the prism in a nearly perpendicular direction with little loss. It is then "totally" reflected from the oblique face, again with very little loss. 3. A third advantage of considerable importance is the stability of the apparatus; it rarely gets out of register. Forty-five degree prisms are made in about three qualities. The most perfectly corrected ones are very expensive; the second or even third grade prisms of the best manufacturers are suitable for camera lucida work.

ILLUMINATION OF THE DRAWING-PAPER

The second modification is that referred to on a previous page as the suspended slide or blind worked by foot-power. (Fig. 1, 32 and f Figs. 2, 3 and 4). This slide enables the operator to illuminate the drawing with almost any degree of light at an instant's notice without disturbing the adjustment of any part of the microscope or camera lucida.. This is highly important in the rapid production of good camera lucida sketches. Especially with high powers, the light coming through

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the microscope is often so faint that it is only by almost completely shutting off the light from the drawing, that the investigator can see at the same time both his pencil and the details of the structures to be sketched. With the foot-power arrangement the operator modifies the light in a second without disturbing the position of his body or his drawing-point, and instantly brings about that adjustment which is most favorable for any particular part of the sketch. Briefly, we may say that the operator's left arm rests on the left-hand "leg-of-mutton" shaped table on a level with the fine adjustment of the microscope, with his left hand in position to work the fine adjustment screw with the greatest ease and accuracy. His right hand, carrying the drawing-point, rests on the drawing board and is engaged in producing the sketch. As the light required for the different portions of the sketch varies he can effect the necessary change in illumination of the drawing paper by a slight movement of his right foot, which disturbs neither his hands nor the equilibrium of the instruments.

METHOD OF TRACING CAMERA-LUCIDA DRAWINGS

As will be at once conceded by any one who makes a trial, black paper is best adapted for camera lucida drawing; a white drawing point should be used. This is an improvement over a pencil used on white paper. The best combination is a thin black tissue-paper, blued on the under side by rubbing on dry prussian blue powder. A piece of drawing paper or enameled board of suitable size for the drawing is pinned to the drawing board,—i. e., the right-hand leg-of-mutton shaped table,—and is then covered with the black tissue-paper, blue side down, pinned at its back edge only. The sketch, a blue tracing, is now made with a fine white ivory or bone point. This blue sketch is put aside for further reference, or for the production of a finished drawing later on, or it may be inked in at once. The object aimed at is a satisfactory representation of the object to be illustrated, of sufficient size to admit of liberal reduction when the ink drawing is photographed on metal preparatory to etching. If it is desired to publish an illustration having a magnification of say 250 diameters, it is advisable to produce a blue sketch of at least 1,000 to 2,000 diameters.

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Highly magnified sketches are easily obtained with the apparatus just described, for by placing the prism reflector at a considerable horizontal distance from the eye-piece of the microscope, say one foot, and lowering the right-hand leg-of-mutton shaped table, magnifications of 5,000 diameters and upward are easily secured. Not infrequently the production of a large coarse drawing is an easier matter than the production of the same drawing on a smaller scale. The conversion of the blue sketch into a pen and ink drawing presents no special peculiarities, but perhaps it ought to be mentioned that the object of using blue is to avoid trouble arising from alterations that may become necessary in finishing the drawing. Any light blue lines which are left on the drawing paper need not be removed, as they do not affect the photographic film sufficiently to cause any inconvenience in the production of an etched block. The black tissue paper mentioned is produced by inking ordinary tissue. The ordinary blue carbon paper gives too dark a blue to meet the requirements. A good quality of blue tracing paper may be made from black typewriter carbon-paper that has been exhausted by the typewriter,—the blue powder being rubbed onto the clean side of the carbon paper.

DARKENING THE ROOM CONTAINING THE INSTALLATION

In addition to blackening all accessories, arrangements are made to darken the room itself, in fact, to make it convertible into a photographic dark-room at will. All the window-blind connections are light-tight. The oblong aperture, about five inches by eight inches, through which the microscope mirror receives its light is screened by means of several thicknesses of flexible black cloth made into the form of a sleeve. This cloth sleeve, attached to the perimeter of the beveled aperture, is slit above and made to surround the microscope just beneath the stage, and the margins overlap and button to one of the screws at the back of the microscope; the lower part of the microscope is thus located within the sleeve. No light reaches the observer's eye except that which comes through the instrument.

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If, now, the slide in front of the large glazed aperture be closed and all direct light shut out, the operator sits in darkness. Any one who has had experience with a photographic dark-room must have observed how after a period of from five to ten minutes therein the eye becomes accustomed to the darkness of the room and is able to distinguish objects much more readily than at first. This is a principle which can be utilized to advantage in connection with high-power microscope work. In fact, it appears to be the relation between the external and the internal illumination which leads so many operators to use artificial light, and even in some cases to prefer working in the evening. If the surrounding light is dim and the eye is allowed to adjust itself to this dimness, then on looking through the properly adjusted microscope, certain details may be seen more clearly than in any other way. It is sometimes painful to witness the unconscious efforts of microscopists to bring about this condition as fully as possible by means of awkward attitudes and facial expression. It is not at all uncommon to see the microscope placed in a glare with strong light beating on the top of the preparation being examined and thence reflected confusingly up through the microscope, and to see the operator sitting in a cramped position, bending his head over the top of the instrument so as to shade his eye-piece as much as possible, and thus prevent eye-piece reflections. All this painful effort is simply an attempt to give the eye the benefit of a weak extraneous light and to prevent confusing reflections from the top of the mount and the eye-piece. With the apparatus here described these difficulties are minimized. The room is darkened. All light which could reach the operator's eye is excluded, except that which comes through the microscope. There is no light falling upon the top of the object, to cause confusing reflections inside the microscope, nor can rays of light be thrown into the eye from the top of the eye-piece, or from high lights on the stand. The image to be examined is as clear as it can be made and the eye is given every facility to see it, and is distracted by no others.

The advantages of this system of using a microscope are not confined to high-powers. It is well known that the central portions of microscope lenses act more perfectly than the peripheral portions.

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By shutting out these latter, better optical results are secured, but the illumination is considerably diminished. The low degree of illumination is less objectionable when the observer is in darkness or semi-darkness. I believe this is due to some extent to relaxation of the iris muscles.

It might at first be thought that this would result in a less perfect image on the retina of the eye, and no doubt this is true if we consider the entire image on the retina. The fact is, however, that the observer concentrates his attention upon a small portion of the retina image and this portion may be focused as accurately as his eye admits. In respect to perfection of image, a comparison of the human eye to an ordinary camera may be very misleading.

CAMERA-LUCIDA DRAWINGS OF DIFFICULT OPAQUE OBJECTS

Camera lucida drawings of opaque objects present numerous difficulties, prominent among which is the small amount of light coming through the microscope. By illuminating the object with a strong light, sunlight if necessary, and reducing to a minimum the amount of light coming from the drawing paper, it is not at all difficult with this apparatus to produce satisfactory drawings of these difficult objects.

Needless to say the apparatus is a daylight apparatus. It hardly seems necessary to argue that as daylight is the light that has developed the human eye it is probably the light to which it is best adapted. This seems a sufficient argument for the use of daylight and a sufficient explanation of its superiority to every other light for the average run of microscopic work. Using the installations here described, monochromatic light of superlative quality is easily obtained by interposing colored glasses or liquids. However, when all this is said, it is not possible always to secure and control daylight so as to get the best results. The following contrivances are such as experience has shown to be very useful for this purpose, especially in sunny climates.

SOURCE OF LIGHT FOR THE MICROSCOPE

Outside of the microscope window a universally adjustable three-by-five-foot white screen is placed in a sunny position, preferably about ten feet away. The surface of this screen should be

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smooth but not shiny and may be of any fine-grained white material. It can be made of wood, painted white, or lined with plaster of Paris; or, what is better, be a plain wooden screen covered with sheet metal and over all several thicknesses of bleached cotton cloth. Whitewash makes a cheap and very excellent white surface for this purpose, and may be applied over cloth. If a small mirror be attached to one corner of the screen, it will indicate the position of the screen that will reflect to the microscope a maximum of white light. Place the screen so that the flash of sunlight from the mirror strikes in the vicinity of the microscope; then the whole screen will be in corresponding position and reflect a maximum of light to the microscope. It is better if this screen can be adjusted from the interior of the microscope room, but this is not essential. After the screen is set the light from it remains for an hour or more practically constant, so that while an adjustment by cords or other mechanism from the interior is a convenience, it is not a necessity. If an adjustable screen is not available it may be possible to arrange two fixed screens, one screen for morning and the other for afternoon.

Blue sky is not a satisfactory source of light. A white cloud gives a very good light, but clouds are so changeable that it is not wise to rely upon them. It is therefore much preferable to construct, as described, an adjustable white screen that will be available whenever the sun shines. When the sun does not shine the sky may serve, or if the day is too dull, one resorts to artificial light.

MICROSCOPE INSTALLATION BOLTED TO THE MASONRY OF THE BUILDING

The installation just described can be bolted to the masonry of the building by means of small vertical I-shaped or Z-shaped girders as shown in Fig. 2. The installation is in most respects similar to that just described, except that the microscope is carried on a heavy cast-iron cross-piece, m, sliding up and down on a pair of L-shaped or Z-shaped girders, and carrying with it an opaque roller blind, u, which passes around a roller at the bottom of the window near the floor.

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In Fig. 2, a number of details are more clearly shown than in Fig. 1, for instance, the details of the headrest, j, which consists of two oblique cork-covered pads, one circular, the other square, each carried on a horizontal screw so that they can be rotated and at the same time moved inward or outward. These pads screw into a cross-piece, which in turn is fastened securely to a vertical rod which slides up and down and rotates, and is clamped by the set-screw shown near k. The great adjustability of these pads makes it easy to fit them to the head of any operator. They serve to keep the observer's eye in register, as well as to decrease fatigue, and actually improve his observing power.

Owing to the action of the heart and lungs, and to the unsteady action of the muscles of the neck, the head, when unsupported,

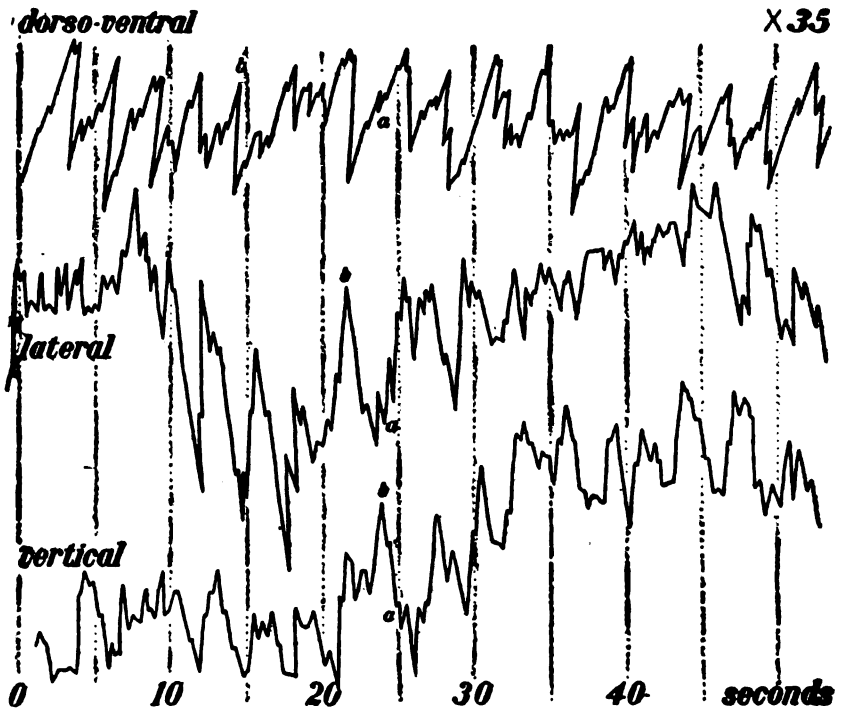

FIG. 5. Three successive graphs showing the nature and extent of the motions of the head of an observer while looking through a microscope. These graphs were obtained by recording the trace of a beam of light from a small mirror actuated by the head of the observer at the microscope.

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is in constant motion, and the image of any stationary object formed meanwhile on the retina of the eye is moving in a corresponding way. The movements of the microscopist's head are shown in the accompanying three graphs (Fig. 5). Both the nature and extent of the rotations about three coordinate axes, at right angles to each other, are shown at a magnification of 35 diameters. These graphs were obtained by recording the trace of a beam of light reflected from a small mirror actuated by the head of the observer at the microscope. The smallest motions indicated in the graphs, a, a, a, are due to the action of the heart; each tiny irregularity represents a heart-beat. The larger irregularities, such as those shown at b, b, b, are due to lung action; the head rises and falls with each breath. Moreover, these two irregularities, those due to the heart's action and those due to the action of the lungs, are distributed on a great curve, which I suppose to be a curve of fatigue connected with the action of the muscles of the neck. These graphs show how complicated are the movements of the image on the retina. Keenness of vision is a function, among other things, of the steadiness of the retina. This is a matter of personal experience, but arguments in favor of a steady retina may be suggested somewhat as follows: It is a matter of common observation that all organisms possessing well-developed eyes, hold the head as steady as possible when looking intently. If while reading from a printed page one wags the head perceptibly, even at so slow a rate as that of the heart's beat, vision is very considerably impaired; the less of this motion, the keener the vision. What is the limit of this improvement in vision due to increased steadiness of the retina? Theoretically it would seem to be reached when the retina is absolutely steady. When we consider the minuteness of the elements in the retina having to do with vision,—the rods and cones and other elements—it seems a very reasonable supposition that vision can be increased in keenness beyond the degree attained by holding the head as steadily as possible by means of the cervical muscles. Whether these arguments are valid or not, the writer is convinced, from years of experience with mechanical headrests, that their use adds to the acuteness of vision. Moreover, they diminish fatigue, making it possible for the observer

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to use the microscope hour after hour continuously with a smaller expenditure of energy.

The attachment of the large prism is also more clearly shown in Fig. 2. It is mounted on a triangular sheet-metal frame, which revolves on a vertical axis, and is clamped in any position by means of the lever shown in the midst of the triangle.

Fig. 3 is an outside view of the installation shown in Fig. 2, and is correspondingly lettered. Note the headrest in use, and also the spectacle lens between the operators' eye and the prism, e. This spectacle lens is one adapted to the operator's eye, and in case he is accustomed to wear ordinary spectacles for reading or drawing, he is able to dispense with them, which is an advantage, as they would be a hindrance in camera lucida work. The substituted spectacle lens should be made by the optician who makes the operator's spectacles, and according to much the same formula; this lens enables the operator to see his pencil point and drawing clearly.

In this illustration is also shown on a shelf in front, a small heliostat, actuated by the small alarm clock, the top of which is to be seen in the figure. The lower reflector of the heliostat is a mirror, the upper a piece of finely matt white card-board. This heliostat may be used instead of the large reflector previously described, and gives a very good light.

INSTALLATION CARRIED OUT IN STEEL TUBING

The installation shown in Figs. 1, 2, and 3 may be carried out in steel tubing as shown in Fig. 4. This form of installation has not yet been thoroughly tried out, but appears to offer a number of advantages over the other forms at an expense but little, if any, greater. The peculiarities of the construction are well set forth in Fig. 4, and as the lettering is the same as in the preceding figures, little need be added. It will be noted that the sash weights are for the most part suspended inside the tubes, and that the various accessories slide up and down on the tubes through the mediation of cast iron rings, malleable castings, turned to fit the tubing somewhat loosely. The microscope is supported on a vertical tube descending

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into another tube of slightly larger size embedded in the cement below, and is counterpoised by a weight which slides in a third smaller tube, also embedded in the cement below. The details are shown in section in the illustration. Collars threaded into the outside tube are turned to fit the tube carrying the microscope. The cross-piece, 1, is suspended on metal straps passing into the spring-pulleys, xx. In all other respects the installation is almost identical with that shown in previous illustrations.

Ordinary rough tubing may be used if its outer surface is smoothed by filing. With such a rough finish, however, the apparatus is liable to be noisy during the adjustment of the various slides. This noise can be eliminated by turning the tubes in a lathe having a long bed, and carefully fitting the cast iron rings to the tubes. With good fitting the apparatus is then practically noiseless. This turning and fitting, however, adds materially to the expense. This installation is neat in appearance, and easy to work. The tubing used is from 3½ inches to 6 inches internal diameter.

This installation differs from those shown in Figs. 2 and 3 in respect to the screen in front of the microscope through which light is admitted to the mirror of the microscope and to the drawing. In the present installation this screen is attached to the tube that carries the microscope, and therefore slides up and down with this tube and with the microscope substantially as shown in Fig. 1. In the tubular installation, therefore, this screen is of very light construction,—made of wood, and very thin,—and its ends, as in Fig. 1, extend deeply into the 10-inch groove at either side of the window.

In Fig. 4 the cloth sleeve that surrounds the microscope has been removed, so as to show the size and nature of the aperture through which the light is admitted to the mirror of the microscope. It will be seen that in this aperture light-filters in the form of glass tanks or colored glasses can be installed *ad libitum*. This method of using tanks and glass filters is a very convenient one. Those of large size can be used.

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The shelf at the top of the installation, carrying the spring-pulleys, x, x, and clamped in position by the higher set-screws, v, v, and supporting the pulley, o, has for its main object to keep the four cylindrical pillars in register,—that is, parallel to one another,—and to prevent vibration.

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MORPHOLOGY OF ADULT AND LARVAL CESTODES FROM POULTRY*

JOHN E. GUTBERLET

During the course of experimental studies on the life history of certain chicken cestodes described in the succeeding section of this work (Gutberlet, 1916) it was necessary to determine exactly the morphological features of the species, which to be sure, had been studied by others but were only partially and imperfectly known. In the former paper I recorded experiments which demonstrated the intermediate stage of *Choanotænia infundibuliformis* to be in the common housefly, *Musca domestica,* and discussed the symptoms of infection and methods of control for tapeworm diseases in chickens.

In this paper are taken up the structure of the adult and cysticercus of *Choanotænia infundibuliformis* (Goeze), and also the adult form of four other species occuring in chickens of this country.

As described more fully in the preceding paper the worms were removed from the intestine under water. The use of normal salt solution was avoided since it was found to be injurious. The cestodes were killed in a corrosive-acetic solution and preserved in 70% alcohol and glycerine. Best results were secured by staining in Delafield's or Ehrlich's acid hæmatoxylin and destaining in acid alcohol.

The five species discussed here were collected at two widely separated points, a farm at Hardy, Nebraska, and the poultry farm at the University of Illinois. These morphological studies were carried on at the Zoological Laboratory of the University of Illinois. This work was taken up at the suggestion of Dr. Henry B. Ward, to whom I am greatly indebted.

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*Contributions from the Zoological Laboratory of the University of Illinois under the Direction of Henry B. Ward, No. 57.

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Structure of Adult and Larva (Cysticercus)

A. ADULT

Choanotænia infundibuliformis (Goeze 1782) Railliet 1896

1. Diagnosis: Length 50 to 200 mm. Scolex (Fig. 2) small, rounded, or conoidal, about 0.4 mm. wide. Rostellum (Fig. 2, 3, *r*) 60 to 70μ in diameter, armed with a single row of 16 to 20 hooks (Fig. 8) 25 to 30μ long, with long dorsal root and short ventral root. Suckers prominent, elongated antero-posteriorly, length 180 to 210μ; breadth 135 to 175μ between the extreme outer edges. Neck short and unsegmented, somewhat narrower than broad. In specimens well extended neck much narrower than head. Anterior proglottids very short and as they become older funnel-shaped, much narrower at anterior than at posterior margins; posterior segments 1.5 to 2.5 mm. broad and 1.5 to 3 mm. long according to amount of contraction, with convex lateral borders, nearly as wide at anterior as at posterior margin. Genital pores irregularly alternating, situated one in each segment in the anterior third of the lateral margin, usually under cover of the backward projecting border of the preceding segment. Vas deferens (Fig. 14, *vd*) and vagina pass between excretory canals and dorsal to nerve trunk.

Male Reproductive Organs: Testicles (Fig. 14, *t*) 25 to 40 or more, 60 in some cases, in posterior half of proglottid, posterior and lateral to large yolk gland, within limits of excretory canals. Vas deferens passes forward and in anterior third of proglottid forms a mass of coils between ovary and excretory vessels from which it extends outward as a convoluted tube to base of cirrus pouch. Cirrus pouch (Fig. 14, 15, *cp*) ovoid in shape, 75 to 95μ in long diameter. Portion of vas deferens in cirrus pouch is much coiled. Cirrus 50 to 65μ long, armed with spines; outer surface of cirrus pouch forms base of deep genital cloaca.

Female Reproductive Organs: Vaginal opening in genital cloaca posterior to cirrus. Vagina posterior to cirrus pouch, after crossing ventral excretory canal dilated to form ovoid seminal receptacle, posterior and ventral to vas deferens, extending to well developed shell gland, 40 to 50μ in diameter located in front of middle of proglottid. Transversely elongated ovary (Fig. 14, *o*)

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occupies anterior portion of middle field of proglottid in front of shell gland. Large yolk gland posterior to ovary and shell gland, irregular in shape, elongated transversely, with convex ventral surface and concave dorsal surface. Uterus (Fig. 16, *u*) developed as tube between anterior and ventral lobes of ovary. Gravid uterus fills up most of proglottid, extending beyond excretory canals on each side. Eggs oval (Fig. 7), with very thin membrane next embryo, followed by thick, smooth membrane 40 by 32μ to 45 by 36μ in diameter, and one or two outer membranes, very thin and wrinkled in preserved material. Diameter of outer membrane 65 by 40μ to 60 by 45μ; at each pole of outer membrane a delicate appendage. Embryonal hooks 18μ long. Embryo 32 by 22μ in diameter.

2. *Morphology:* The scolex of the living worm shows up very prominently and can be used as a distinguishing feature. When first removed from the intestinal wall the suckers appear distinct and the neck is much narrower than the scolex. Soon after the removal it often contracts and takes on the appearance of a flattened bulb which includes the neck and anterior segments (Fig. 1). This feature is characteristic of this species and is a factor which alone assists very materially in distinguishing it from others that occur in chickens.

The rostrum or crown of the scolex is somewhat pointed when the rostellum is enclosed within its sheath (Fig. 2). The rostellum is an ovoid structure with a bulbous expansion at its anterior end. It has a length of 140μ and a breadth of 60 to 65μ at its anterior end. A crown of 18 hooks is arranged in a single row around the bulbular anterior end. The structure of the wall is of a fibrous nature and presents a transversely striated appearance due to contraction. In the interior of the rostellum the structure is a connective tissue mass with few cells, some of which possess long processes. The hooks (Fig. 8) are 30μ in length with a long dorsal root and a short ventral root.

The rostellar sheath or sac (Fig. 3, *rs*) into which the rostellum is withdrawn is oval in shape and 230 to 240μ in length by 80 to 90μ in width at its broadest point. Histologically, the structure is that of a fibrous connective tissue type with spherical and spindle-shaped cells. The cells coming in contact with the rostellum, as

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well as those on the outer edge of the sac, bear long processes. The outer layer of the rostellar sac is composed of longitudinal and oblique fibers of a muscular nature which probably have for their function the movement of the rostellum.

The four excretory canals, that have extended forward through the entire length of the body, unite in the scolex to form a ring (Fig. 3, *ex*), which lies in the tissue of the rostellar sac around the body of the rostellum.

The suckers are prominent. They are oval in shape and in preserved specimens measure 180 to 210μ in length and from 135 to 175μ in extreme breadth. In the center of each sucker there is a depression or an acetabulum, 30 to 40μ in diameter. The entire inner surface of the suckers possesses minute hooklets or spines (Fig. 4) 1.5 to 2μ long. These hooklets not only line the suckers but also extend over the entire surface of the scolex (Figs. 3, 5) and down onto the neck region; they disappear before reaching the first segment. They appear more distinctly on scolices that are somewhat contracted than on those that are well extended. These hooklets can be seen only in sections as they are too small to be distinguished readily in whole mounts.

Musculature: The longitudinal muscle fibers are arranged in bundles which are scattered, forming a loose irregular layer. The bundles are numerous and nearly of a uniform size. There are no transverse muscle fibers present except a few minute oblique fibers which connect some of the longitudinal fibers near the ends of the proglottids. Some dorso-ventral fibers are present, but they are not abundant.

Nervous System: The longitudinal nerve fibers are arranged in fiber tracts which approach the structure of a nerve cord. The individual fibers do not form a compact mass, but are more or less free in the tract. Nerve cells have no definite arrangement, but are situated irregularly along the fiber tract (Fig. 6). The nerve cells are somewhat spindle-shaped and quite large, being from 20 to 25μ long by 6 to 8μ wide with large nuclei. Transverse nerves are composed of individual cells with long processes extending transversely from the lateral fiber tracts. The transverse fibers are much scattered and have no definite arrangement except that they

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are more numerous near the ends of the proglottids. Peripheral nerve cells are widely and irregularly distributed. They are more numerous at the anterior end of the proglottids, especially on the portion that is covered by the backward extension of the preceding segment.

Excretory System: The excretory system is fairly well developed in this form. The ventral canal (Fig. 14, *v ex*) is the larger, and has a diameter of 28 to 30μ. A transverse canal unites the two longitudinal canals in each segment. The dorsal canals (Fig. 14, *d ex*) are much smaller, having a diameter of 6 to 8μ and are not united by transverse connections. The four longitudinal canals extend anteriorly to the scolex where they unite to form a ring which lies in the rostellar sheath around the body of the rostellum. The vas deferens and vagina pass between the dorsal and ventral excretory canals.

Male Reproductive Organs: The testes vary in number, usually from 25 to 40, but in a few cases the number is much greater, being as high as 55 or 60. The testes are quite large, being from 40 to 55μ in diameter, and are located in the posterior half of the proglottid (Fig. 14, *t*), posterior and lateral to the yolk gland. The testes are not arranged in layers, but are grouped in a more or less compact mass almost entirely within the limits of the excretory canals. The vas deferens (Fig. 14, *vd*) in the anterior third of the proglottid forms a coiled mass at the side of the ovary, from whence it passes laterad to the cirrus pouch as a convoluted tube. The portion of the vas deferens inside the cirrus pouch is coiled, varying in extent in different specimens (Figs. 14, 15). The vas deferens passes into the cirrus. There is no seminal vesicle formed by the vas deferens in the cirrus pouch nor are there any accumulations of sperm cells. The cirrus pouch (Fig. 15) is ovoid in shape and is from 75 to 90μ in diameter. The wall is made up of layers of fibers which are both circular and oblique, forming a basket-like network which incloses the cirrus and a portion of the vas deferens. The outer wall of the cirrus pouch forms the inner wall of the deep genital cloaca. The cirrus is a compact structure from 50 to 65μ long and lined with spines. It is a slightly curved structure passing from the cirrus pouch and curving posteriorly

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toward the vagina which is directly posterior to it. The cirrus was not observed extending from the genital cloaca, but was noted in some specimens curving toward the vagina, though not passing into it. A few sperm cells were present in the vas deferens, also in the vagina and the seminal receptacle.

Female Reproductive Organs: The large ovary (Fig. 14, *o*) lies in the anterior third of the proglottid and extends transversely across the segment. It has a length of 300μ and a breadth of about 75 or 80μ at its broadest point. It is irregular in shape, being composed of a number of lobes. The end which is nearest the genital pore is smaller than the other, allowing room for the mass of coils of the vas deferens, the vagina, and the seminal receptacle. The ovary is concave on the dorsal surface and convex on the ventral. On the dorsal surface of the end nearest the genital pore is located the seminal receptacle and the vagina. The ova are large and very distinctly shown in the ovary (Fig. 16). Posterior to the ovary is the large yolk gland (Fig. 14, 16, *y*) which lies about the middle of the proglottid. It is irregularly elongate in shape and extends transversely across the segment, having a length of from 120 to 130μ and a breadth of from 35 to 50μ. Immediately in front of and dorsal to the yolk gland and posterior to the ovary is the shell gland (Fig. 14, *sg*) which is slightly ovoid in shape, 40 to 50μ in diameter. A small duct, the vitelline duct (Fig. 16, *v*), passes from the yolk gland through the shell gland from which it receives a duct. The combined ducts after passing through the shell gland unite with the oviduct (Fig. 16, *ov*) which appears as a curved tube leading from the ovary. These united tubes or ducts pass anteriad and slightly ventrad into the uterus which develops as a blind tube in the region of the ventral lobes of the ovary. This blind tube (Fig. 16, *u*) grows in size and extends transversely across the segment. As it becomes larger the tube forms pockets which extend anteriorly and posteriorly and also dorsally, until it takes up the entire mass of the proglottid between the excretory canals. In gravid segments it even extends beyond the excretory canals. A small tube or duct, which is really the end of the vagina, connects the seminal receptacle with the yolk-shell gland duct and oviduct. This tube serves to carry the sperm to the eggs in the oviduct for fertilization. The

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seminal receptacle (Fig. 16, *sr*) is a dilation of the vagina into an oval shaped structure which is about 50μ long and from 25 to 30μ in breadth at the widest part. From the seminal receptacle the vagina passes laterad, lying posterior to the cirrus pouch, and unites with the genital cloaca. The genital cloaca has its pore on the lateral margin near the anterior end of the proglottid. The pore is usually covered by the backward projection of the segment anterior to it. The vas deferens and vagina pass between the dorsal and ventral excretory canals and dorsal to the nerve tract. The vas deferens is dorsal and anterior to the vagina.

In the mature segments the uterus becomes filled with ova and it increases in size until it occupies the entire area between the excretory canals, even extending beyond the canals in the gravid proglottids. The uterus finally breaks up into compartments, each containing a single embryo. The embryos (Fig. 7) are about 32 by 22μ in diameter with onchospheric hooks 18μ long. Usually three membranes, but often four, enclose the embryo. The inner membrane is thin and closely surrounds the embryo; the next is heavy, being from 1.5 to 2μ thick, composed of fibrous layers with a few cells present. This layer is variable in thickness, depending considerably upon the amount of contraction of the segment, as it ranges in size from 40 to 32μ to 50 by 36μ, or it may be even slightly larger. Usually one (Fig. 7) and sometimes two thin membranes are found on the outside of the thick layer. These are often wrinkled and bear at each end an appendage formed from the outer membrane by which it is attached to the wall of the capsule or compartment of the uterus.

In this species the oldest proglottids drop off from the worm before they are fully mature. The embryos from the oldest segments on the worm do not show the characteristics of entirely mature ones, and there are distinct differences between them and those that have been separated from the worm for some time. Single proglottids that have separated from the worm are quite active and remain in the intestine for some time before passing out with the feces. Proof of this is furnished by the fact that a large number of the free proglottids are found in the intestine at any time. Even tho only a few worms are present in the intestine of a bird there is

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usually a large number of free proglottids. If they did not remain in the intestine for a considerable length of time there would not be nearly as many. Further proof is furnished by the fact that the free proglottids have embryos which are mature, showing the onchospheric characteristics, while the oldest segments that are still attached to the worm have embryos that are not entirely mature. This same condition has been observed in *Davainea proglottina* as Blanchard (1891:435) states that the oldest proglottids separate from the others and remain in the intestine to become mature before passing out. The proglottids do not always separate from the worm singly, but may drop off in groups of three or four.

The fact that the proglottids separate from the worm before they are entirely mature is one of great importance in taking up experimental work for infection of intermediate hosts. If the embryos are fed to insects or other invertebrates before they are mature they will be digested, and thus infection cannot be produced.

B. CYSTICERCUS

The cysticercus of *Choanotænia infundibuliformis* was found in the abdominal region of the body cavity in the common house fly, *Musca domestica*. The flies had been fed on embryos from ripe proglottids of this species of worm, and at the end of twelve days were killed. The cysticerci appear to be nearly ripe or ready for transmission into the adult host. The time for the development of the cysticercoid varies with different species and under different conditions. Grassi and Rovelli (1892:85) found that *Davainea proglottina* developed from the onchosphere into a ripe cysticercus in less than twenty days. Schmidt (1894:9) found that the development of the cysticercoid of *Drepanidotænia anatina* (Krabbe) varied with the time of the year and the influence of the temperature. In the summer the embryo developed in an ostracod, *Cypris ovata*, into ripe cysticercoids in two weeks.

The cyst proper (Figs. 11, 12, *c*) containing the scolex is oval in shape, 220μ long and 120μ in diameter.

The bladder (Fig. 12, *b*) or tail, which is also oval in shape, is located against one side of the cyst and is somewhat flattened on that side. It is 220 to 230μ long and from 116 to 120μ in breadth.

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The scolex is 80μ in breadth and 120μ in length; neck is 40μ in diameter and 30 to 35μ long; suckers are 55 to 60μ in diameter. The rostellum is 60μ long and 20μ in breadth, armed with a crown of 18 hooks arranged in a single row. These hooks (Fig. 9) are 30μ long with a long dorsal root and a short ventral root. The suckers are lined with numerous minute hooklets or spines 1.5 to 2μ long which extend over the edges of the suckers and also over the greater part of the surface of the scolex, including a part of the neck region. Schmidt (1894: 16) described cuticular hooklets on the suckers of *Drepanidotænia anatina*.

The size of the scolex may be somewhat variable as shown by those in the cysticercoids of *Drepanidotænia anatina* by Schmidt (1894: 10). In that species the intermediate host could be one of two or more species of crustaceans and the size of the cysticercoid varied with the size of the host in which it was parasitic.

The head of the rostellum is conical in shape, bearing a bluntly pointed apex anterior to the end of the dorsal roots of the hooks (Fig. 10, *r*). This part of the rostellum is composed of minute muscle fibers which are both circular and oblique. The rostellum is slightly broader below the circle of hooks as it is an oval shaped body.

The rostellar sac (Fig. 10, *rs*) is a deeply stained structure 10 to 12μ thick. It extends from 10μ below the hindermost part of the rostellum to the anterior extremity of the scolex, forming an oval shaped sac or sheath. It is composed of parenchymatous tissue with large heavily stained oval or spindle shaped cells which bear processes. The outer part of the sac is composed of a thin layer of fine fibers which help to give it a definite shape. At the lower edges of the sac the fibers are connected or associated to some extent with similar fibers that form the inner layer of the suckers. The anterior region of the rostellar sac, which forms the sheath for the free head portions of the rostellum, is constructed of an inner layer of fine fibers and an outer layer of large spindle-shaped cells, the most of which bear fibrous processes at one or both ends.

The suckers are composed of large spindle-shaped cells which are arranged perpendicular to the edge. These are heavily stained

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and form a compact layer. The inner boundary of the suckers is composed of a layer of fibers which are both circular and oblique. Some of these at the upper edges are associated with similar fibers in connection with the rostellar sac.

The cyst is composed of two cell layers with an irregular cavity between them. The cells are large and irregular in shape with no special arrangement in the layer. Large intercellular spaces lie between the cells, thus forming a loose network structure, except at the base of the neck. At this point where the neck is attached to the inner layer of the cyst the cells are smaller and are in a compact mass. There is no definite boundary to the outer part of the inner layer as well as to the inner part of the outer layer of the cyst. Few cells with long connective processes extend across the cavity from one layer to the other. This then forms an irregular cavity (Fig. 11 *ca*) 2 to 20μ in width between the two layers of the cyst. This is the primitive cavity of Grassi and Rovelli (1889: 373). The two layers of the cyst are formed apparently by a fold which extends upward and inward from the base of the neck, forming the gastrula cavity of Grassi and Rovelli (1889: 402, *g*) and enclosing the scolex. This cavity varies in width from 3 to 10 or 15μ.

The bladder, an oval shaped structure, is located at one side of the cyst and is attached to it at the posterior end by a narrow connection (Fig. 12, *cn*). The posterior end of the cyst or the region caudad of the base of the neck is somewhat drawn out (Fig. 12). From this point is given off the attachment to the bladder or tail portion of the cysticercoid. The fact that this bladder is really a tail, even though it possesses a cavity, is shown by the presence of the onchospheric hooks, which are located at the end of the bladder opposite to that of the attachment of the cyst (Fig. 12, *oh*).

The order of arrangement of the onchospheric hooks is individual. In some specimens they are situated at the end of the bladder, while in others they are at the side. In some the arrangement is in a group, while in others they are in pairs. Some of my specimens show a pair of embryonic hooks in the layers of

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the cyst between the base of the neck and the attachment of the bladder, while the other two pairs of hooks are located in the bladder.

The cavity of the bladder is formed apparently by a splitting or hollowing out of the cells of the tail, because the wall is continuous and of the same histological structure. The wall of the bladder is constructed of two layers, an inner cell layer and an outer cuticular layer. The outer cuticular layer is more or less striated on account of minute fibrils uniting it with the inner cell layer. Histologically, the structure of the inner layer is constructed of somewhat granular substance arranged in fibers forming a network which encloses clear spherical cells with large nuclei (Fig. 13). Outside of the cuticular layer is located the peritoneum of the host which lies upon the bladder and surrounds it as well as the cyst.

C. COMPARISON OF ADULT AND CYSTICERCUS

A comparative study of the adult and the cysticercoid shows the likeness which exists between them. The presence of the same number of hooks, having exactly the same size and shape as seen by comparing Figures 8 and 9. Minute hooklets of the same size are present in both cysticercoid and adult lining the suckers, the entire surface of the scolex and a part of the neck region. Rosseter (1891: 365) shows that the hooks on the rostellum and suckers of *Echinocotylus Rosseteri* undergo no changes during the act of transition from cysticercus to adult stage. The rostellar sac is of the same general shape in both. The head of the rostellum is not expanded in the cysticercoid as in the adult because it has not functioned as yet. This corresponds to figures as shown by Schmidt (1894, Pl. VI, Fig. A) of the cysticercoid and Krabbe (1869, Pl. VI, Fig. 114) of the adult of *Drepanidotænia anatina,* and by Grassi and Rovelli (1892, Pl. IV, Fig. 7, 8) of the cysticercoid and Blanchard (1891: 16) of the scolex of *Davainea proglottina.* No measurements are given for the rostellum of either the cysticercoid or the adult by the above authors.

There is a great deal of difference in the size of the scolex between the cysticercoid and the adult. In my specimens the

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scolex of the adult is between four and five times as large as that of the cysticercoid. The scolex of the cysticercoid has as yet not functioned so that the musculature of the organs is not developed as in the adult, consequently is not nearly as massive. The cells also are smaller than those of the adult.

Schmidt (1894: 10, 44) shows that the adult scolex of *Drepanidotænia anatina* is about three times as large as that of the cysticercoid. He also states that the size of the cysticercoid may vary with the size of its host.

Different forms become modified in changing from the intermediate to the adult hosts as shown by Schmidt (1894) in *Drepanidotænia anatina*, Rosseter (1891) in *Echinocotylus Rosseteri*, and Grassi and Rovelli (1892) in *Davainea proglottina*.

Onchospheric hooks in the wall of the tail are the same size (18μ) and shape as those of the embryos found in the mature proglottids.

A consideration of these factors of morphological significance which demonstrate the resemblances between the cysticercoid and adult, indicates clearly that this cysticercoid is the intermediate stage of *Choanotænia infundibuliformis*.

OTHER CHICKEN CESTODES IN THE UNITED STATES

1. *Davainea tetragona* (Molin 1858) Blanchard 1891

Diagnosis: Length 10 to 250 mm. by 1 to 2.5 mm. in breadth, varying with state of contraction. Scolex (Fig. 19) 175 to 215μ in diameter, with retractile rostellum 25 to 50μ in diameter, armed with single row of about 100 hooks. Rostellar hooks (Fig. 20) 6 to 9μ long through longest axis, hammer-shaped, with long ventral root and short dorsal root, prong short and recurved. Suckers oval, 60 to 110μ in diameter, armed with 8 to 10 rows of small hooks of various sizes. Acetabular hooks (Fig. 21) range in size from 4 to 8μ through longest axis, having thorn-like prong, short dorsal root, and longer flattened ventral root, which is shorter than prong. Neck long and slender, but often as broad as head. Segments trapezoidal and imbricate, edges of strobila serrate. Oldest segments usually longer than broad, often bell-

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shaped. Genital pores usually unilateral, situated one in each segment, at or in front of middle of lateral margin, frequently marked off by papilla. Male and female canals pass on dorsal side of nerve and excretory vessels.

Male Reproductive Organs: Testes 20 to 30 in median field surrounding female organs, most of them lying on aporose side of latter. Vas deferens situated in anterior third of segment, beginning near median line, and extending in much convoluted course laterally to base of cirrus pouch which it enters and, after a few coils in basal portion of latter, passes into cirrus. Cirrus pouch pyriform, 75 to 100μ in length. Basal portion surrounded by prominent layer of longitudinal muscle fibers, neck with thick layer of transverse fibers. Cirrus without apparent spines.

Female Reproductive Organs: Ovary in middle of segment. Yolk gland posterior to ovary, irregularly reniform, slightly longer in its transverse axis, about 100μ in diameter. Shell gland prominent, 50μ in diameter, immediately in front of yolk gland. Vagina begins at genital pore, posterior to opening of cirrus pouch, at first very slender but at distance of 15 to 25μ from genital pore swells out into thick-walled tube, functioning as seminal receptacle. This extends transversely across segment and joins oviduct on dorsal side of ovary near median line. Oviduct, after being joined in shell gland by vitelline duct, proceeds forward and ends on dorsal side of ovary. Definite and persistent uterus not developed. Eggs pass from distal end of oviduct, become imbedded in fibrous and granular or gelatinous mass which fills up most of segment. This mass divides into 50 to 100 portions to form egg capsules, each surrounded by membrane and containing 6 to 12 or more eggs. Egg is surrounded by three envelopes,—inner, close to onchosphere, often scarcely visible; middle layer or envelope much folded, giving appearance of network between inner and outer membranes; and smooth outer envelope. The onchosphere measures 10 to 15μ in diameter; the outer envelope measures from 25 to 50μ in diameter.

One point noted here that has not been mentioned before by other authors is that the genital pores are irregularly alternate.

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They are usually unilateral. The existence of this irregularly alternate occurrence of the genital pores may be an anomaly, but it is rather frequent for such a condition.

2. *Davainea echinobothrida* (Megnin 1880) Blanchard 1891

Diagnosis: Length up to 250mm; width 1 to 4 mm. Head (Fig. 22) 0.25 to 0.45 mm. in diameter, with retractile rostellum 100 to 150μ in diameter, armed with crown of about 200 hooks arranged in two rows. Suckers round or oval, 90 to 200μ in diameter, armed with 8 to 10 rows of hooks. Rostellar hooks (Fig. 23) similar to those of *Davainea tetragona,* but larger, measuring 10 to 13μ in length. Acetabular hooks (Fig. 24) likewise similar to those of *D. tetragona,* but also larger; size variable, smallest being 7 or 8μ in length and largest measuring from 14 to 16μ. Neck thicker and generally shorter than *D. tetragona,* nearly equal to width of head. Strobila resembling that of *D. tetragona,* but serrate border more pronounced. Oldest segments in preserved specimens also differ from those of *D. tetragona,* being less elongate and frequently marked by median constriction. Owing to this constriction adjacent borders of most posterior segments pull apart in median line and remain joined only at sides, giving rise to median series of openings through posterior portion of strobila. Genital pores irregularly alternate, or sometimes almost entirely unilateral, situated one in each segment posterior to middle of lateral margin. Male and female canals pass on dorsal side of nerve and excretory vessels.

Male Reproductive Organs: Testes 20 to 30, arranged in median field surrounding female glands as in *D. tetragona.* Vas deferens lies in anterior third of segment much as in *D. tetragona.* Cirrus pouch flask-shaped, 130 to 180μ in length. Basal portion globular or ovoid, surrounded by layer, about 10μ thick, of longitudinal muscle fibers inside of which is a layer about 12μ thick of transverse fibers. Neck of pouch measures 50μ to 75μ in length by 15 to 20μ in diameter, surrounded by layer of transverse fibers thickened at distal end to form sphincter. According to Mégnin, the cirrus is armed with minute spines.

Female Reproductive Organs: Female organs same as in *Davainea tetragona,* and onchospheres (Fig. 25) are also similar

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in structure and size, 14 to 15μ in diameter. Onchospheric hooks 6 to 7μ long. Egg capsules in groups of 6 to 12 or more, embedded in a fibrous gelatinous mass.

In the living specimens very little difference can be noticed except in size of the species *D. tetragona* and *D. echinobothrida*. They are both quite transparent and appear much alike in every respect in external appearance, except that *D. tetragona* is slightly more transparent, while the oldest segments of *D. echinobothrida* have very distinct median constrictions between them, appearing almost as a series of openings.

The chief differences between *D. tetragona* and *D. echinobothrida* are that in the latter the animal is larger, the hooks are more numerous and larger, and the structure and size of the cirrus pouches show a very distinct difference. There is also a difference in the pathological effect of these spiny-suckered forms. *D. echinobothrida* produces large nodules or ulcers in the intestinal wall. The scolex bores through the mucosa of the intestine and in some cases nearly through the muscular coats. This disease in fowls is termed "nodular tæniasis", as described by Moore (1895: 1), and is often mistaken for other diseases.

3. *Davainea cesticillus* (Molin 1858) Blanchard 1891

Diagnosis: Length 10 to 125 mm. Maximum width 1.5 to 3 mm. Head cylindrical (Fig. 28), sometimes spheriodal, 0.3 to 0.6 mm. wide and 0.2 to 0.4 mm. long. Suckers unarmed, about 100μ in diameter. Rostellum broad and flat or hemispherical, 0.25 to 0.35 mm. wide, armed with a crown of 200 to 300 hooks which are very unstable and easily lost, arranged in two ranks. Hooks (Fig. 29) 8 to 12μ long with short dorsal root and long ventral root. Neck very short. Anterior segments three to five times as broad as long; the following increase in size until they become equal in length and breadth and finally even longer than broad; borders overlapping. Genital pores irregularly alternate, one in each segment, somewhat in front of middle of lateral margin in young segments and nearer the middle in older segments. Vagina and cirrus pouch pass dorsal of the two excretory canals and nerve.

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Male Reproductive Organs: Testes (Fig. 17, *t*) 20 to 30 in number in posterior portion of segment. Vas deferens much coiled before entering base of cirrus pouch, also coiled within latter. Cirrus pouch ellipsoidal, 120 to 150μ long by 55 to 70μ wide. Cirrus when protracted 10μ in diameter, armed with minute spines, and with bulbous enlargement 20μ in diameter at its base, where it becomes continuous with cirrus pouch.

Female Reproductive Organs: Vagina enlarged before reaching median line into small seminal receptacle (Fig. 17, *sr*). Ovary occupies middle field in front of testes. Yolk gland and shell gland posterior to ovary, ventral and dorsal, respectively, in relative position. Uterus at first in front of ovary as cord of cells; gradually increasing in size, finally occupies most of segment and frequently extends laterally beyond excretory canals. In oldest proglottids it becomes divided into compartments, or capsules, each containing a single egg. Embryo (Fig. 30) 36 by 27μ in diameter, with very thin membrane closely adherent to surface. Embryo further enveloped by thicker, smooth fibrous membrane, oval in shape, 45 to 40μ in diameter, with filament at each pole attaching to thin outer wrinkled membrane about 35 by 50μ in diameter: finally egg is surrounded by capsule composed of outer and inner membrane, latter closely adherent to or fused with outer egg membrane; and former more or less widely separated from latter and connected with it by number of septa.

One of the principal points noted here that is not mentioned by other authors is the size of the rostellar hooks. In my specimens they seem to be somewhat larger than those described by others. They have been described as being 8 to 10μ long, while my forms show many of them to be distinctly 12μ in length. A second point noted here is the method of the development of the uterus. The uterus develops in front of the ovary. It first appears as a solid cord of cells connected with the united ducts of the ovary, shell gland, and yolk gland. The solid cord of cells which later gives rise to the uterus becomes hollow and appears as a blind sac or tube. This then grows in size, forming pockets, and finally fills up the entire proglottid.

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This form is one of the most common chicken tapeworms and is the most easily recognized. It can be identified by the head with its broad, flat rostellum which shows up very prominently; the width of the most anterior segments is usually equal to or greater than the width of the head, and the eggs are distributed in individual egg capsules in mature proglottids.

4. *Hymenolepis carioca* (Magalhaes 1898) Ransom 1902

Diagnosis: Length 30 to 80 mm. Breadth at neck 75 to 150μ, at posterior end 0.5 to 0.7 mm. Segments three to five times or more broader than long throughout strobila. Head (Fig. 26) flattened dorso-ventrally, 140 to 160μ long, 150 to 215μ wide and 100 to 140μ thick. Suckers shallow, 70 to 90μ in diameter, unarmed. Rostellum unarmed; in retracted position 25 to 40μ in diameter and 90 to 100μ in length, with small pocket opening to exterior in anterior position. Unsegmented neck portion of strobila 0.6 to 1.5 mm. long. Genital pores almost entirely unilateral, a single pore being located in each segment slightly in front of middle of right-hand margin.

Male Reproductive Organs: Testicles three in number, normally two on left and one on right of median line. On dorsal side of inner end of cirrus pouch vas deferens is swollen into prominent seminal vesicle (Fig. 18, *sv*) which may attain a size of 70 by 50μ. Cirrus pouch (Fig. 18, *cp*) in sexually mature segments 120 to 175μ long by 15 to 18μ in diameter; almost cylindrical, slightly curved toward ventral surface of segment; on outer surface about 20 longitudinal muscle bands, 2 to 3μ in thickness, very prominent in cross section; vas deferens enlarged within cirrus pouch to form small seminal reservoir occupying proximal two-thirds of pouch; distal third of portion of vas deferens within pouch very slender, about 1μ in diameter and functions as cirrus. Genital cloaca 12 to 36μ deep.

Female Reproductive Organs: Opening of vagina in floor of genital cloaca, ventral and posterior to cirrus opening. First portion of vagina very narrow, 1μ in diameter. Small vaginal sphincter 8 to 10μ from vaginal opening. On inner side of sphincter vagina gradually increases in diameter, and in sexually mature

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segments swollen into prominent seminal receptacle (Fig. 18, *sr*) which extends forward to anterior border of segment and inward considerable distance beyond proximal end of cirrus pouch. Ovary faintly bilobed or trilobed in posterior half of proglottid. Yolk gland spherical or ovoid, 30 to 40μ in diameter, situated near median line of segment, posterior and dorsal of ovary. Uterus at first solid cord of cells extending transversely across segment along anterior border of ovary; becomes hollowed out and grows backward on dorsal side of ovary; in gravid segments occupies nearly entire segment and filled with eggs. Eggs (Fig. 27) in gravid uterus spherical or oval, with four thin membranes, the two middle membranes often approximate to form thick layer which shows somewhat of a cellular or coarse granular structure. Diameter of outer membrane 36 by 36μ to 75 by 70μ, of outer middle membrane 30 by 30μ to 65 by 60μ, of inner middle membrane 26 by 26μ to 40 by 35μ, of inner membrane 24 by 16μ to 29 by 21μ. This membrane often lies so close to onchosphere that it can scarcely be distinguished from edge of embryo. Onchosphere is 18 by 14 to 27 by 19μ in diameter; length of embryonal hooks 10 to 12μ.

This form is thread-like and usually occurs in great numbers. It is very delicate and fragile and can be recognized by that fact alone, as it is the most fragile of the chicken forms known.

SUMMARY

1. By morphological comparison of the cysticercoids produced experimentally in flies and adult of *Choanatænia infundibuliformis* they are shown to be identical.

2. Morphological points noted are the presence of minute hooklets on the suckers and entire surface of scolex in *Choanatænia infundibuliformis*. The manner of development of uterus in the same species is by means of a blind tube which grows in size, forming pockets, and later breaks up into small compartments. In *Davainea tetragona* the genital pores were found to occur irregularly alternate in the proglottids. The hooks on the rostellum of *Davainea cesticillus* were found to vary in length from 8 to 12μ. The uterus in development first appears as a solid cord of cells which becomes hollow and in growing forms pockets, filling the entire proglottid.

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EXPLANATION OF PLATES

Unless otherwise stated all drawing were made with the aid of a camera lucida.

Abbreviations

b—bladder	*rs*—rostellar sac
c—cyst	*sg*—shell gland
ca—primitive cavity	*sr*—seminal receptacle
cn—connection of bladder with cyst	*sv*—seminal vesicle
cp—cirrus pouch	*t*—testes
des—dorsal excretory canal	*u*—uterus
es—excretory ring in scolex	*v*—vitelline duct
o—ovary	*va*—vagina
oh—onchospheric hooks	*vd*—vas deferens
ov—oviduct	*ves*—ventral excretory canal
r—rostellum	*y*—yolk gland

Plate V

Choanotaenia infundibuliformis

Fig. 1. Scolex much contracted. x40

Fig. 2. Scolex normal extension. x145

Fig. 3. Longitudinal section of scolex, showing rostellum and rostellar sac. x425

Fig. 4. Section of portion of sucker, showing hooklets. x425

Fig. 5. Section of portion of wall of scolex, showing hooklets. x425

Fig. 6. Longitudinal nerve tract, showing nerve cells with processes. x650

Plate VI

Fig. 7. A, B, C, D. Embryos from mature proglottid. x425

Fig. 8. Hooks from rostellum of adult. x425

Cysticercus of Choanotaenia infundibuliformis

Fig. 9. Hooks from rostellum of cysticercus. x425

Fig. 10. Section through scolex, showing rostellum with hooks and rostellar sac. x425

Fig. 11. Section through scolex and cyst, showing suckers with hooklets, structure of cyst and primitive cavity between layers of cyst. x425

Fig. 12. Reconstruction of cysticercus with cyst and bladder or tail, showing scolex in cyst and onchospheric hooks in bladder. x145

Fig. 13. Section of wall of bladder, showing histological structure and peritoneum of host. x425

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PLATE VII

Fig. 14. Choanotaenia infundibuliformis. Reconstruction of mature proglottid, showing reproductive organs, excretory vessels, and nerve. x145

Fig. 15. C. infundibuliformis. Reconstruction of cirrus pouch showing cirrus and vas deferens, also part of vagina in connection with cloaca. x310

Fig. 16. C. infundibuliformis. Reconstruction of female reproductive organs, showing part of ovary, yolk gland, shell gland, oviduct, vitelline duct, uterus, and connection of ducts with uterus and seminal receptacle. x310

Fig. 17. Davainea cesticillus. Reconstruction of mature proglottid, showing reproductive organs and excretory vessels. x145

Fig. 18. Hymenolepis carioca. Reconstruction of mature proglottids, showing reproductive organs from ventral view. x145

PLATE VIII

Fig. 19. Scolex of Davainea tetragona. x145

Fig. 20. Hooks from rostellum of D. tetragona. x425

Fig. 21. Hooks from suckers of D. tetragona. x425

Fig. 22. Scolex of Davainea echinobothrida. x145

Fig. 23. Hooks from rostellum of D. echinobothrida. x425

Fig. 24. Hooks from suckers of D. echinobothrida. x425

Fig. 25. Embryos of D. echinobothrida, showing capsule and fibrous gelatinous mass in which it is embedded. x425

Fig. 26. Scolex of Hymenolepis carioca, after Ransom.

Fig. 27. A, B, C, D. Embryos of Hymenolepis carioca, showing enveloping membranes. x425

Fig. 28. Scolex of Davainea cesticillus. Free-hand drawing of living specimen well extended, showing rostellum.

Fig. 29. Hooks from rostellum of D. cesticillus. x425

Fig. 30. A, B, C, D. Embryos of D. cesticillus, showing enveloping membranes. x425

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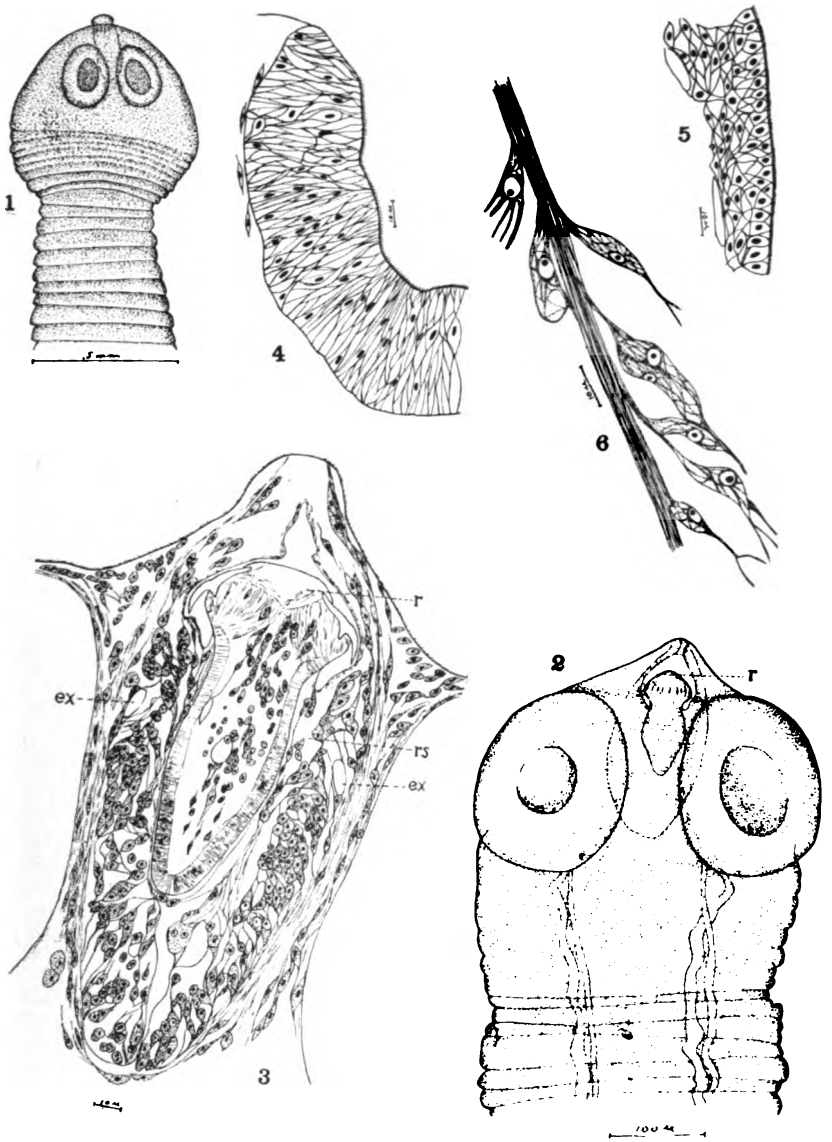

PLATE V

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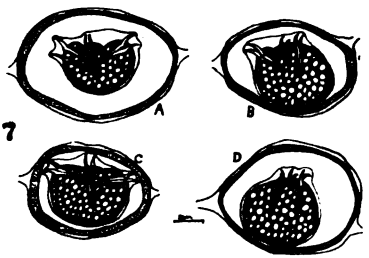

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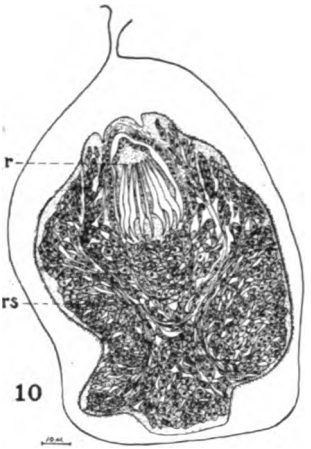

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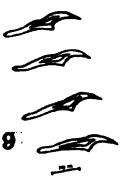

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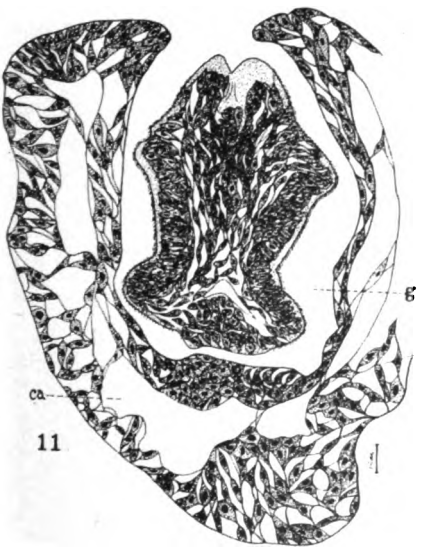

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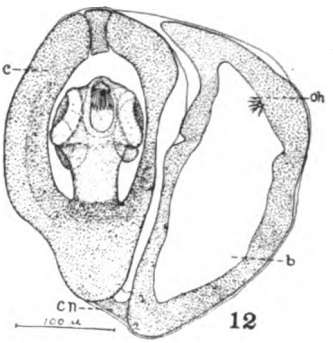

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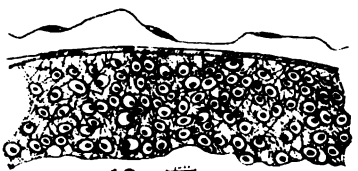

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PLATE VI

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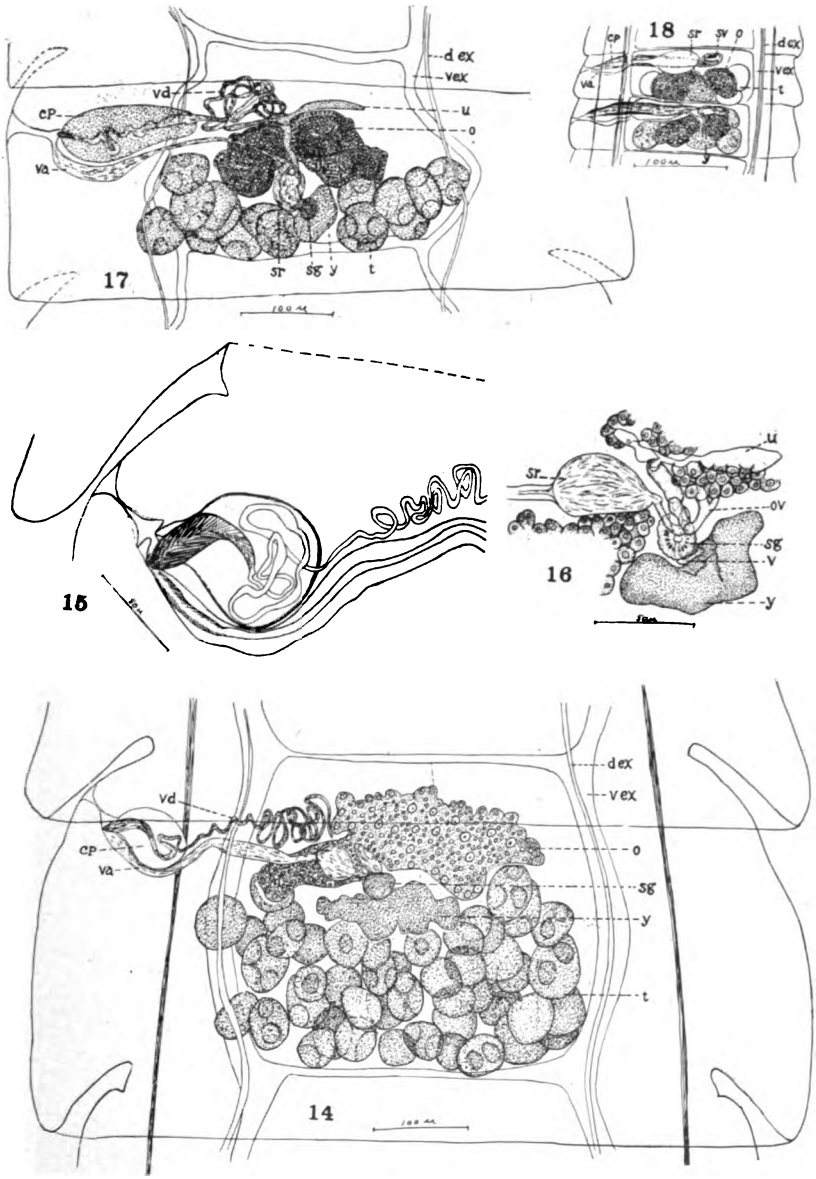

PLATE VII

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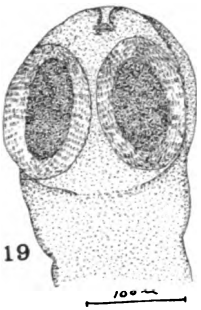

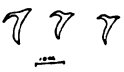

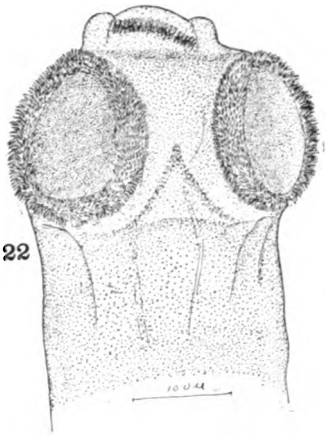

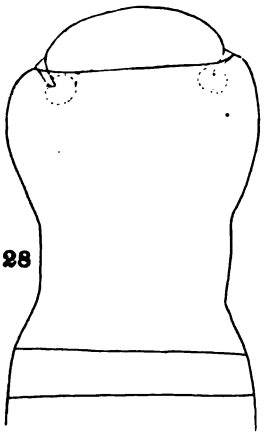

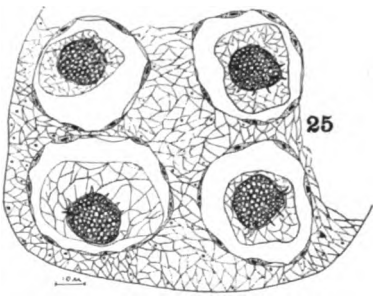

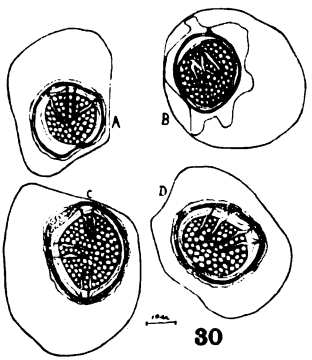

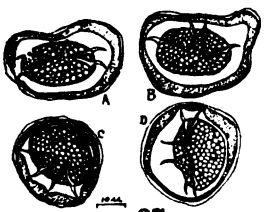

PLATE VIII

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A PRELIMINARY STUDY OF THE SPERMATOGENESIS OF BELOSTOMA (ZAITHA) FLUMINEA

By A. M. Chickering

The study of *Belostoma fluminea* was begun during the summer of 1914 at the University of Wisconsin under the direction of Prof. M. F. Guyer to whose help and criticism the writer owes much. I also wish to take this opportunity to express my gratitude to Prof. H. D. Densmore of Beloit College for the loan of expensive and indispensable apparatus.

MATERIAL AND METHODS

Belostoma fluminea is the common, giant water bug of our shallow ponds and slowly moving streams. This form is very common in the region around Madison, Wisconsin and has been taken in large numbers, especially during the month of July.

Nymphs and adult males, which are often taken bearing the egg-cluster on their backs, were used to obtain the necessary material. Some of the adults show the whole general history of the germ cells from spermatogonia to mature spermatozoa with great clearness. By careful study the seriation is placed beyond reasonable doubt by virtue of the arrangement of the cells in cysts in a nearly progressive series from one end of the testes to the other.

On account of their extreme delicacy and transparency some trouble was experienced at the beginning in removing the testes from their position in the abdominal cavity to the fixing fluid. Later, however, this was overcome by squirting the fixing fluid directly on to the testes after removal of the dorsal abdominal wall. After a few moments the organs stood out sharply and could be transferred easily to the final fixing fluid without injury.

The best results in fixing were obtained with Gilson's and Bouin's fluids.

The iron-hæmatoxylin method of staining has proven very satisfactory for this work. Other stains have been tried with

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varying degrees of success but all the slides used in preparing this paper were stained with Haidenhain's iron-hæmatoxlyin. Sections were cut from four to six μ in thickness.

Smears have been almost uniformly unsuccessful. In a few cases smear slides of fair quality were obtained but as yet no careful study has been made of them. The method of preparation as described by Morse ('09) gave better results than any other. The testes are simply removed to a slide and the cysts pricked or teased gently. This allows the contents to run evenly over the slide which is afterwards dried and stained without the use of any fixing fluid.

The works cited at the end of this paper are only those which have been especially helpful to the writer in preparing this brief paper. No attempt has been made to append a complete list of papers on this subject.

SPERMATOGENESIS

I. Spermatogonial stages

The spermatogonial divisions are very numerous in this material, particularly so in the testes taken from nymphs. But usually the chromosomes are so crowded together in these division stages as to prevent any accurate counting or study of the separate chromosomes. In fact the writer has been able to find only four metaphase plates in which the chromosomes could be effectively studied. These were all found in the testes of a single specimen and are probably undergoing final division before synapsis.

The two cells shown in figures 1 and 2, have the chromosomes arranged in a very flat equatorial plate in a plane almost parallel to the plane of the section. Both show twenty-four chromosomes, and in each case there are four large bean-shaped chromosomes, eighteen of intermediate size and spherical or ellipsoid in shape, and two very small ones. Nearly all are connected to one or more of the others by means of delicate filaments.

The other two cells mentioned above show, respectively, twenty-three and twenty-two chromosomes. In the first case one of the small pair is missing and in the second both are missing. These are so small and the chromosomes so numerous that they

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may be covered up easily and so escape detection. From the number and behavior of the chromosomes in the maturation divisions one would expect the spermatogonial or unreduced number to be twenty-four. The writer considers it fair to conclude from the evidence at hand that such is the case.

It is quite evident from inspection of figures 1 and 2, that three pairs of chromosomes may be identified at this stage. The xy-pair, which becomes so apparent in the second maturation division, is not distinguishable nor so far has it been possible to recognize more pairs of chromosomes in this stage.

II. Synaptic and Post-Synaptic Stages

It has thus far been found impossible to obtain satisfactory results with *Belostoma* in attempts to work out the history of the chromosomes during synapsis and the early prophase stages of the primary spermatocytes; hence no attempt will be made to describe these in any detail, only a brief outline being given. I hope, however, to obtain suitable smear material at a later date and make a careful study of the stages necessarily slighted now.

Immediately following the last spermatogonial division the chromosomes loosen up and flow together into a rather lightly staining, confused network (Fig. 3). This stage is of short duration, passing quickly into what is probably a spireme stage but since no suitable material is available no drawings have been made from this point to synizesis (Fig. 4). The threads making up the synaptic knot are so crowded and tangled that usually only the ends of the threads can be seen projecting beyond the heavily stained mass. The knot is usually placed at one pole of the nucleus and often a plasmosome is visible in these stages.

After the synizesis stage the threads quickly spread apart throughout the nucleus. They now appear very thick and heavy, and stain more deeply than in the preceding stages (Fig. 5-6). The plasmosome attains its maximum size during this stage and gradually diminishes and finally disappears during the early prophases.

In the later part of this period some nuclei appear to show a divided condition of the separate threads even in sections and in the best smears the threads are distinctly divided by a longitudinal cleft (Fig. 7). Usually in the sections there are shown

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two threads which are much longer and broader than any of the rest, presumably corresponding to the two large chromosomes of the first division and made up of the four bean-shaped spermatogonial chromosomes.

No evidence has yet been adduced to show that the sex-chromosomes in *Belostoma* are present in the form of chromatic nucleoli during the growth period as described by Wilson ('12) in the spermatogenesis of *Lygæus* and *Oncopeltus* although the general history of the chromosomes seems to be very similar to that of the two forms mentioned.

Before the prophase stages begin there is interpolated a "confused" stage which results from a fading out and ultimate disappearance of the heavy, divided threads. This process gives rise to a nucleus which is traversed throughout by a loose network of chromatin material consisting of much finer, irregular threads the boundaries of which can not be made out. This goes on from Figure 8 until the network is reduced to an exceedingly confused condition. In this condition the threads stain so lightly and are so interlaced as to make it impossible to see the separate elements at all. This "confused" stage is of relatively long duration, but finally condensation starts and the network is reorganized into the tetrad rods, rings and V's (Fig. 9-9A).

III. Chromatoid Bodies

In the confused stage are *clearly* seen for the first time (further work seems likely to disclose their presence earlier) bodies which have been called the chromatoid bodies (Wilson '13 and Fasten '14).

The larger of these bodies which becomes very prominent in the division of the primary spermatocyte attracted my attention when the work on *Belostoma* was first started. It then seemed to be an x-chromosome and for a time the work was carried on with that idea. The reader will readily see the resemblance of figures 14-23 to several already published by numerous workers demonstrating the presence of an odd chromosome. In the case here presented this idea must be abandoned. The chromatoid bodies are seen in Fig. 8 outside of the nuclear membrane and therefore

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not directly connected with the chromatin elements. They are never found within the spindle although they are sometimes so close to the group of chromosomes that they are indistinguishable in polar views of metaphase plates, and in this way sometimes are very confusing in taking chromosome counts. They do not occur in all cells and when present behave irregularly. With iron-hematoxylin they are stained like the chromosomes.

IV. Primary Spermatocytes

In the early tetrad stages of the chromosomes two rings are very large and prominent. They are in practically every cell and seem to give rise to the two large chromosomes of the mature spermatocyte by a decrease in the diameter and a closing up process. There are other smaller rings sometimes showing but they do not seem to be constant. The rods seem to shorten and thicken to form the final chromosomes; the same process probably takes place with the Vs, and the rings apparently close up to form such tetrads as shown in Figs. 10-11. Such figures are very common in my material and may be favorable for working out the details of the changes taking place here.

All of these figures finally condense into dumb-bell shaped bodies (Fig. 12). This shape is retained throughout the first division, the constriction marking the plane of cleavage.

Very shortly after the stage shown in the last figure the nuclear membrane begins to break down, the spindle forms at the two poles, the chromosomes take up a position in the equatorial plane and the cell is ready for division.

The chromatoid bodies remain of about the same size until the spindle begins to be formed and then a decided increase in the size of at least one of them is plainly seen (Fig. 13-15.)

The stages from this point onward show with diagrammatic clearness. There are thirteen chromosomes in the equatorial plate of the primary spermatocyte. This number is one more than half the spermatogonial number. The arrangement of chromosomes is inconstant, no two plates showing the same placing with respect to each other. There are two large, ten intermediate and one very

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small chromosomes, they all divide equally in this division, carrying over thirteen in every case to the secondary spermatocytes (Figs. 14-24).

These stages have been carefully searched to find the sex-chromosomes and to determine their behavior but without success.

The chromatoid bodies are seen here (Figs. 14-24) at their maximum size. Usually there is only one present, but quite often there are two and more rarely three. In a few cases four have been seen. Occasionally one or more of the number has an irregular shape (Figs. 14, 20 and 22).

When division takes place these bodies are not apportioned evenly but may all be retained in a single secondary spermatocyte, or one or more may go into each. No regularity seems to obtain in the distribution of these bodies. Given three or four of different sizes and a large number of types of secondary spermatocytes may be derived if classified on the basis of the kind and number of chromatoid bodies they possess.

V. The Interkinesis

It seems to be a well established fact that in the interkinesis there is no more than a slight pause between the telophase of the first and the prophase of the second divisions. The chromosomes are much crowded together, but enough is evident to show that they retain their individuality and are not in any degree reorganized into a nucleus (Fig. 25). The centrosomes also divide in late anaphase and are already well moved apart, accompanied by small spindles, in the late telophase.

Whether the grouping of chromosomes is changed in this stage is uncertain, but the probability is that the new grouping assumed in the next metaphase takes place during the prophases of the same division.

VI. Secondary spermatocytes

In the prophases of this division the spindle is very rapidly built up while the chromosomes which were so crowded before now spread apart and assume *a grouping very different from that in the first division.* Eleven of the chromosomes are arranged in a circle at the equator of the spindle while two remain in the center

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of the circle forming an xy-pair of sex-chromosomes. In polar views only twelve chromosomes are usually visible (Figs. 26-28). Lateral views of the same stage show however, that what appears to be a single chromosome in polar views is in reality the xy-pair, the members of which are united end to end. (Fig. 29).

All of the stages through inter-kinesis leading up to the metaphase in which the sex-chromosomes are seen united have been studied to see how complete the conjugation between the two is effected, and such a process seems to be limited to a simple end to end union as described above. This union exists only for a short time before the final separation and seems to take place while the chromosomes are rearranging themselves in their new grouping during the later prophase stages.

By a comparative study of Figs. 23-25, and 29-30, it will be seen readily that a rotation of each chromosome through about ninety degrees takes place while the spindle is being formed, so that the long axis of each becomes parallel instead of perpendicular to the long axis of the spindle. A rotation of the entire group may also take place as described for *Oncopeltus* and *Lygæus* (Wilson '10) although it seems more probable that the virtual rotation is accomplished by the relative change in position of the separate chromosomes as they assume their new grouping.

In the latter part of this period the sex-chromosomes have separated and are on their way to the opposite poles of the spindle before the others have even started to divide at all (Fig. 30). This aptitude for the sex-chromosomes seems to be a common phenomenon and has been described by numerous workers.

The chromatoid bodies present about the same appearance as in the primary spermatocytes. There are many cells without any chromatoid body, many more with only one large one, and others with two or three.

VII. The Spermatids

When division of the secondary spermatocytes takes place twelve chromosomes are delivered to each spermatid, but one-half of them receives eleven ordinary chromosomes plus the x-chromo-

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somes while the other half receives eleven plus the y-chromosome (Figs. 32-34).

In studying the cysts containing spermatids it is very evident that those containing no chromatoid bodies are most numerous, those with one fairly common and those with two or more are much less common. In general, too, the size of these bodies is somewhat less than in the primary spermatocyte although occasionally one large one seems to be retained throughout at its maximum size (Figs. 35-38).

VIII. Summary

1. The spermatogonial number of chromosomes in *Belostoma* (Zaitha) *fluminea* is twenty-four.

2. Only general facts have been determined in regard to synapsis and the post-synaptic stages. During the post-synaptic period a double nature of the chromosome threads is evident.

3. There is a "confused" stage just previous to the prophases of the first division.

4. The chromatoid bodies appear in this "confused" stage for the first time. Proof that they originate from the cytoplasm is not lacking because they are plainly seen outside of the nuclear membrane in this stage.

5. Tetrads appear in the form of rings, Vs, and rods, and all become dumb-bell shaped, by continued condensation, at the time that they enter the spindle.

6. The first maturation division is an equational division. Polar views show thirteen chromosomes, which number is one-half the spermatogonial number plus one.

7. The chromatoid bodies are at their maximum size in this and the following division and generally grow smaller from that point onward.

8. The interkinesis is of short duration. No nuclear vacuole is formed, the chromosomes maintaining their individuality throughout.

9. When the chromosomes arrange themselves in the metaphase of the second division an entirely new arrangement is assumed and an xy-pair of sex-chromosomes can be identified.

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10. Twelve chromosomes are delivered to each spermatid in the second division, one-half receive in addition to the eleven ordinary chromosomes an x- and the other half a y-chromosome.

11. The chromatoid bodies behave irregularly all along. Some spermatids have none, others have one and still others in decreasing proportions have two or three.

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EXPLANATION OF PLATES

All figures are from *Belostoma* (Zaitha) *flumínea* are drawn with a camera lucida and are magnified about 1350 diameters. Unless otherwise stated the figures are not intended to show *all* the nuclear components.

PLATE IX

1-2. Polar views of spermatogonial metaphases, showing twenty-four chromosones.

3. Spermatogonial telophase (probably the last spermatogonial division).

4. Synizesis stage with a plasmosome.

5-6. Post-synaptic nuclei showing the heavy undivided chromosome threads and plasmosomes.

7. A little later stage showing the divided threads. From a smear-preparation. The cell is somewhat distorted.

8. The confused stage, showing three chromatoid bodies outside of the nuclear membrane.

9-9A. About middle prophase of the first spermatocyte division, showing the various forms of the chromosomes during condensation, and the chromatoid bodies.

10. Later prophase of the same division, showing the tetrads and only one chromatoid body.

11. A very clear tetrad.

PLATE X

12. A late prophase of the first division showing the chromosomes organized into dumb-bell forms.

13. Very late prophase, showing all the chromosomes and two chromatoid bodies.

14-17. Polar views of metaphases of the first division, showing thirteen chromosomes and either one, two, or no chromatoid bodies.

18-21. Lateral views of the same stage with a varying number of chromatoid bodies.

22-23. Anaphases of the first division illustrating the irregularity in the behavior of the chromatoid bodies during division of the cell.

PLATE XI

24. Telophase of the first division. Here the two secondary spermatocytes have each received one chromatoid body. The four small bodies are the centrosomes already divided preparatory to the next division.

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25. Polar view of the same, showing the crowded condition of the chromosomes.

26-28. Polar views of secondary spermatocytes. All the ordinary chromosomes are here arranged in a circle with the sex-chromosomes in the center and chromatoid bodies without the circle.

29-30. Lateral views of the same, showing the xy-pair of sex-chromosomes in the center.

31. Same stage without the sex-chromosomes.

32. Anaphase of the second division, showing the x- and y- chromosomes going into different spermatoids and slightly in advance of the others.

33-34. Polar views of the same, showing the small y- and the larger x- chromosomes in different spermatids. These cells were somewhat tilted so that in drawing the sex-chromosomes are made to appear displaced from their normal position.

35. Polar view of a spermatid with the chromosomes crowded in telophase, and two chromatoid bodies.

36. Lateral view of the same stage showing one spermatid receiving three chromatoid bodies and the other, none.

37-38. Later views of spermatids just before metamorphosis starts.

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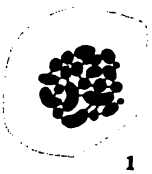

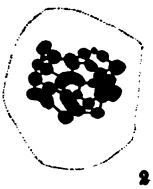

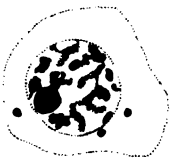

PLATE IX

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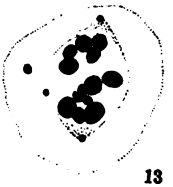

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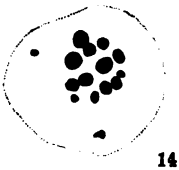

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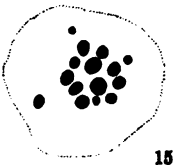

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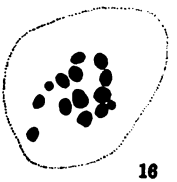

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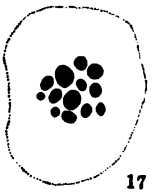

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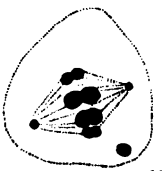

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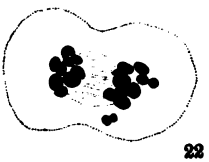

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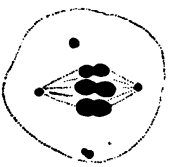

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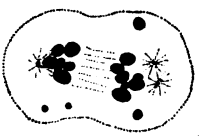

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PLATE X

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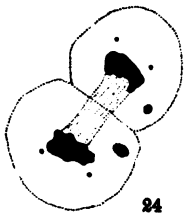
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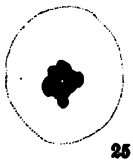
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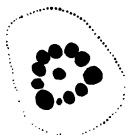
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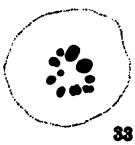
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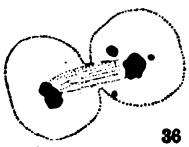
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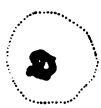
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PLATE XI

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated student suggestions of suitable fields of investigation.—[Editor.]

A SYSTEM FOR RECORDING CYTOLOGICAL MATERIAL, SLIDES AND LOCATIONS ON THE SLIDES

The following contribution is offered in full recognition of the fact that many cytologists already have in use excellent methods of recording their material and slides. Indeed many of the most essential details of the present system of recording slides have been taken over from a method in use by McClung, for which privilege the writer acknowledges his indebtedness. The system to be described has stood the test of the writer's use in all particulars and it is offered here in hope that it may serve as a suggestion for cytologists, who as yet have no recording method, on which to base a system serving their own particular needs. If the scheme here outlined is impractical for certain workers this note will at least serve to indicate the requirements that a cytological recording method must meet to be really efficient.

Two sets of cards are used, one to record the gross material and the other the slides and locations on the slides. On the former card (Fig. 1) are all the notes concerning the material from the fresh condition until it is embedded. On this card is to be found:

1. the serial lot number.
2. the material, the animal or plant from which it was taken, the age and other notes of possible interest.
3. place of collection and condition of obtaining the material.
4. dates of fixation.
5. fixing fluid and the temperature the fluid was used at.
6. time in fluid.
7. washing and dehydration.
8. clearing methods used.
9. embedding methods and materials.
10. location of embedded material.

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Under "Dehydration" in Fig. 1 the time the material remains in each grade of alcohol is recorded beneath that grade. In case the more recent practice is used (not yet published) of displacing the water with alcohol drop by drop an arrow is drawn, as indicated, to the percentage of alcohol the material is in at the end of the displacement. If the tissue was preserved in 70% alcohol then an arrow would be drawn to "70%" and later when the dehydration is continued a second arrow would be drawn to the grade at the

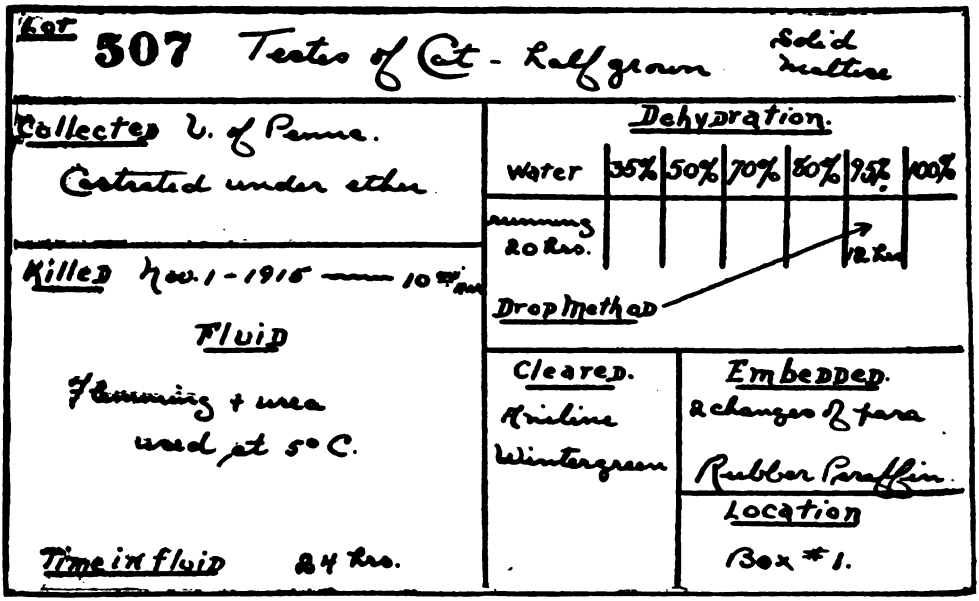

Fig. 1

end of the series. The writer embeds his material immediately as in the long run it saves considerable time and tissue in paraffin is much easier to carry about the country than in bottles. The material under each lot number is usually embedded in a petri dish (the lot number on a small piece of paper is embedded with the material). The disk of paraffin after removal from the petri dish is wrapped in wax paper and filed in a 3x5 cardboard filing case behind an index

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card bearing the lot number. In this way the material is compact and easy to get at.

On the second set of cards is to be found:

1. lot number.
2. slide number.
3. number of box in which slide is located.
4. stain used.
5. what is to be found on the slide.
6. thickness of sections.
7. condition of slide (i. e. good fixation or stain).
8. location of favorable areas on the slide.
9. notes concerning certain locations.
10. areas that have been drawn.
11. areas that have been photographed.
12. areas that have been drawn or photographed and used for publication.

The number is scratched on each slide of the series. If spermatogenesis is being worked upon one card is devoted to a single phase in the process found on a particular slide. Similar phases on other slides have their own cards. This card is labeled as shown in Fig. 2 under "Shows". The same slide may therefore have several cards devoted to it should it show more than a single phase. These cards are filed first behind an index card bearing the lot number and then in numerical order behind index cards bearing the phase name of the particular stage they happen to represent. When the observations do not deal with spermatogenesis then, of course, the cards are classified according to the special need.

As can be seen in Fig. 2 there is a place for forty readings. The right hand reading of the mechanical stage is placed above the short line, the horizontal reading is put beneath it. The slide is first searched with a low power lens and readings of apparently favorable locations are put down. Afterwards these locations are tested with the oil immersion lens and either crossed out or drawn. When the figure is drawn the location is circled as shown in Fig. 2. Any notes that are to be made are indicated by the figures in the space to the right of the readings. These numbers refer to corresponding numbers on the back of the cards under which the notes are written. Small sketches may also be put in these spaces

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to recall what the reading is of. When the plates have been prepared for publication the figure number is entered in the square opposite the reading. When the cell has been photographed this information is also placed here with the number of the photo-

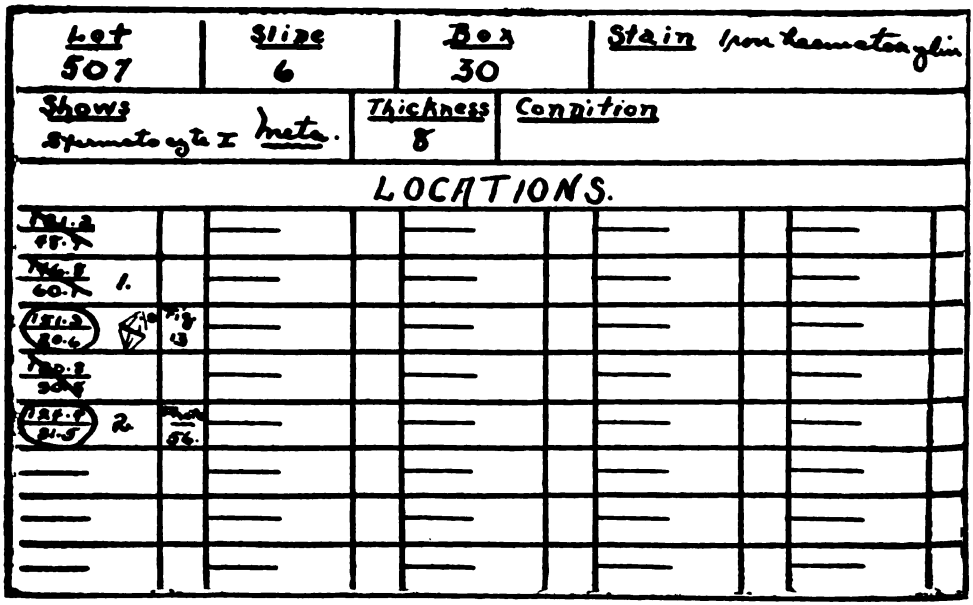

Fig. 2

graph. With these records should the plates be lost or when the original of a figure is to be examined the location on the slide may readily be found.

With the records on these cards before him the investigator has all his data well in hand for the preparation of his paper.

Zoological Laboratory, ROBERT T. HANCE.
University of Pennsylvania.

A MINIATURE DARK ROOM FOR USE WITH THE MICROSCOPE

All microscopists prefer to work either at night or in a darkened room. Using the microscope under such conditions does away with the strain to which both the observing and the unused eye are subjected by the side light—i. e., light coming from sources

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other than through the tube. When working in darkened surroundings the effect is that of looking at a picture on a screen. The image appears brighter and objects become clear that under the usual conditions are scarcely visible.

For several years the writer has been trying to devise some method to control the light perfectly and to do this without necessitating the darkening the whole room. It is desirable that any apparatus for the purpose should weigh little and (for ease in carrying from one place to another), it should be simple to take apart. It should, of course, be adaptable to every condition. For further convenience of the worker definite places should be present in such an apparatus for the usual microscopical accessories—pens, pencils, drawing and memorandum cards and lens paper.

The following description is of a miniature dark room for use with the microscope fulfilling these requirements. It was designed and made by the writer last fall and, after a year's use, he has found it to be exceedingly practical in eliminating all the strain that results when the eye is unshielded. In this darkened enclosure the eye not in use is at perfect rest. Moreover for drawing the light may be controlled so that it is possible always to have light of the same intensity directed on the drawing paper.

Description

Figure 1.

A. Base—¼-inch white pine 12x18 inches with a binder of the same wood across each end to prevent warping.

B. Uprights—dowel sticks 1 inch in diameter cut to 18 inches in length.

C-C′. Rods—common telescoping curtain rods. Each of the rods C′ is cut 8 inches from the end that ordinarily would be used to fasten it to the window. C is formed of the remainder, of the part between the ends.

D. Wire—a piece of annealed wire ⅛ inch in diameter about 4½ feet long bent as shown.

To assemble:—one two inch screw fastens each upright to the base. The upright on the right can be seen to have two angle irons aiding in its support but this is only necessary when the fan is

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added. Holes are drilled in both uprights to correspond to the diameter of C which is inserted in them. The rods C′ are attached by one end to the tops of the uprights by a screw through the eyelet in the rod. Through the eyelet at the opposite end a small rod is passed as shown to prevent the curtains from slipping off. The wire D is fastened to the outer sides of the uprights by means of a single round head screw passed through each flattened end. All the wood and metal work is painted a dead black.

For many valuable suggestions on the design of the curtains and for the excellence of their construction I am indebted to my mother. (See figure 2).

The curtains suspended from the rods C and C′ are in four parts, all overlapping each other and fastening together with spring snaps. They are made of the heaviest grade of black sateen doubled. On the right hand curtain are pockets for pencils and cards. On the left side is a pocket for lens paper. The pocket is provided with a flap to exclude the dust. The upper curtain carried on the wire D is of single thickness. The central curtain is in two parts so that they may be separated to permit light to fall on the drawing board. The left hand curtain of the central set has a rectangle 1 inch wide by 5 inches high cut from the center of the basal portion. Across the top of this aperture is stitched a flap of double thickness, 3½ inches wide by 5½ inches in length. To one corner of the loose end of the flap is attached a tape which passes around the tube of the microscope and fastens to the other corner of the flap by means of a spring snap.

With the microscope surrounded by these curtains it is impossible to read the figures on the mechanical stage and so the small light (fig. 1 E) was installed. This can be adjusted by means of sliding rods locked with winged nuts to hang directly over the stage. The lamp arm is attached to the right hand upright by means of a collar made of two pieces of brass stripping fastened on either side of the pillar with a thumb screw. The lamp is a small tungsten bulb set in a porcelain socket. The shade or reflector, shown in the photograph, was taken from an old tubular flash light. A small three-cell pocket battery furnishes the current which is

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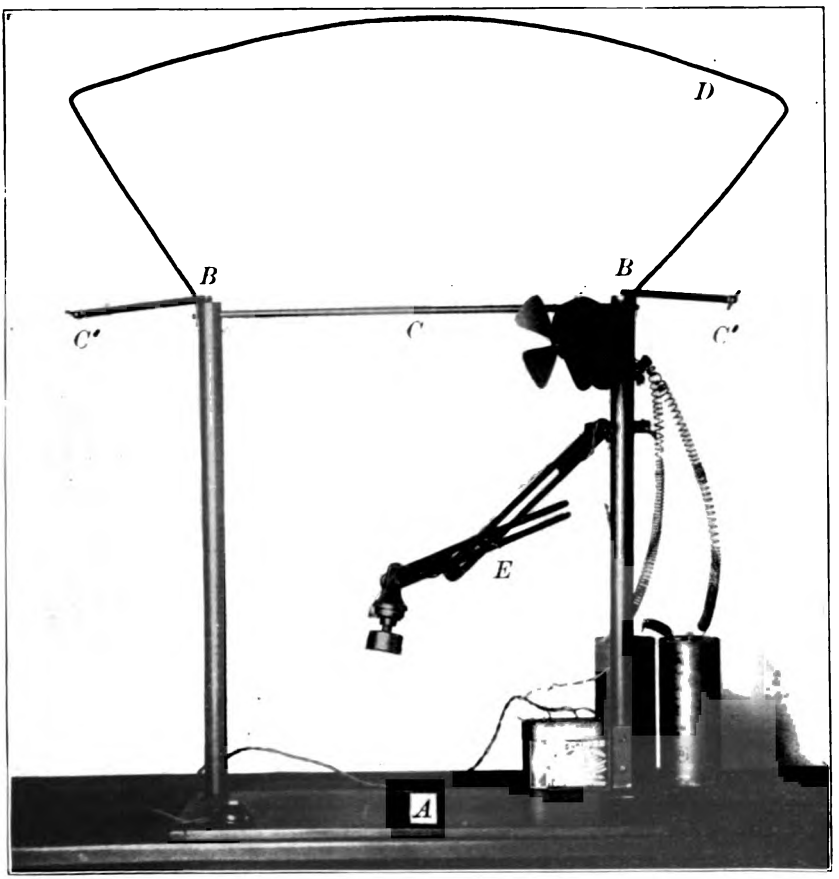

PLATE XII

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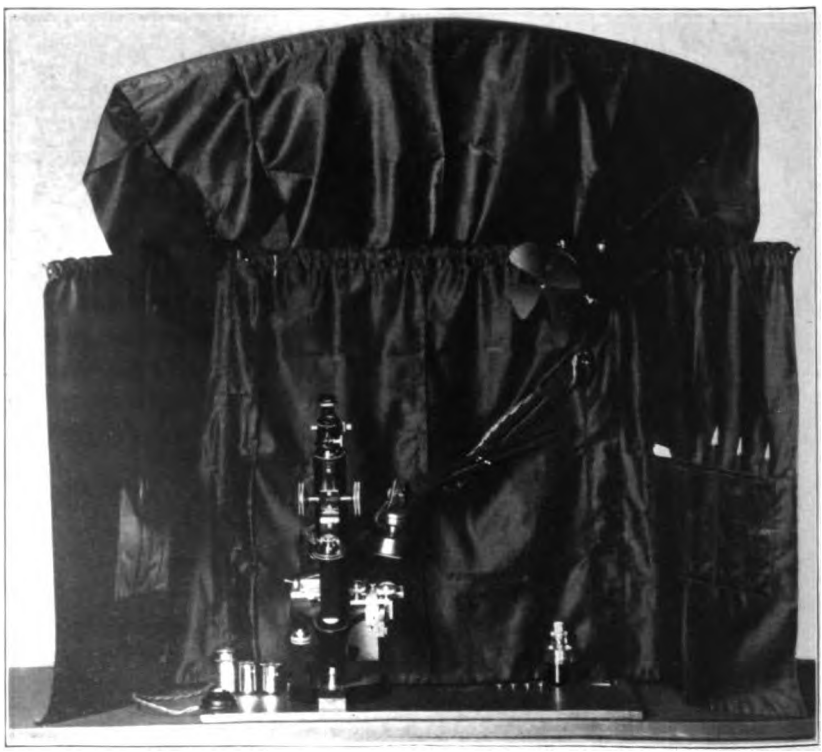

PLATE XIII

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controlled by a push button at the left of the microscope. The same battery has lasted for very nearly a year now without visible signs of weakening.

The fan shown in both photographs is a toy motor equipped with a 4½ inch blade. The motor is operated on two dry cells. It is fastened to a wooden base that is inserted in a slot in the upright and clamped tight by means of a winged nut. This fastening permits the fan to be tilted up and down while the single screw securing the fan to the base allows a left and right rotation. The air current may thus be directed on any spot desired.

Operation

For microscope illumination with this dark room a concentrated filament mazda frosted globe is used. This globe is placed behind the slit in the central curtain and the microscope is put in position on the opposite side. The flap covering the slit is then snapped about the tube of the microscope just above the nose piece. The slit through which the light comes is so narrow that the stage of the microscope effectively shields the eye from the light coming through the lower part of the slit while the flap takes care of all other dispersion.

In the average room having windows on only one wall the side curtains can be left wide apart. In places where the worker is almost surrounded by windows it is of advantage to draw the side curtains so close that there is just room for the observer's head to enter. The telescoping rods supporting the side curtains permit these to be narrowed or widened to suit the circumstances. The top curtain works to or from the microscopist and is frequently convenient in cutting out the light from the upper parts of the windows.

Light on the drawing paper is obtained by separating the lower portions of the central curtains from each other and fastening them back. The bulb illuminating the microscope then throws its light over the right hand side of the base. A constant intensity of illumination is in this way assured.

The fan is a luxury—possibly an unnecessary one, but in very warm weather or on days when a few flies persist in maintaining

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their position at all hazards on top of the writer's head he has not been at all skeptical as to whether the luxury was unnecessary or not.

Zoological Laboratory, ROBERT T. HANCE.
University of Pennsylvania.

NOTES ON A NEW SPECIES OF LOXODES (EHRBG.)?

In the course of work upon the distribution of fresh-water protozoa in the southeastern part of Massachusetts many species were found which could not be named according to the available classifications. This is true of various species of the genus Loxodes.

Loxodes belongs to the class of Infusoria and to the sub-class Ciliata; that is, the protozoon is provided with cilia or setæ during all of its stages, but is free of flagella. This sub-class is divided into a number of orders, Loxodes falling under the order of Holotricha. This order includes the ciliata which possess but one kind of cilia and show the anus and mouth conspicuously. The members of the genus Loxodes show a hook-like projection on the anterior end which is bent to the left, and cilia cover nearly the entire body. The body is flattened, slightly elongated and possesses a well defined outer envelope of the cell or ectoplasm which is constant in form. The dorsal surface is free from cilia, smoothed and curved. The ventral surface is flat and well ciliated, with a mouth on the left anterior edge which is at the bottom of a slit-like peristome. Some writers claim this leads into a pharynx, the existence of which I have not been able to see. The animal is a free swimmer and shows nuclei clearly.

The species under consideration has an average length of *60* microns and width of *16* microns. It is found in great numbers among Oscillaria, associated with *Nassula, Paramecium* and rotifers. There was no evidence of its feeding upon the algæ. Its food consisted principally of small paramecia.

They may be narcotized for study by a .47% solution of cocaine hydrochloride; though after about two minutes the animal slowly assumes an oval shape, then becomes round and all evidence of life ceases. The action of the narcotic was not so pronounced up-

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on the paramecia and rotifers, for at the end of ten minutes locomotion was evident in these, although not so rapid as in the clear water.

During swimming these ciliata have a spiral motion which is to the right. Trichocysts were noted in the larger specimens, this being limited to the posterior region. Some of them seemed to possess contractile vacuoles while others did not. The anterior end is hook-like but not a rostrum. In this respect it differs from *Loxodes rostrum* (Mull.); the anterior end is also more blunt, and the dorsal surface was more curved than in *L. rostrum.*

Further study upon this species and the genus is in progress.

Chadwick, N. Y. ELTON R. DARLING.

ENTOMOLOGICAL NOTES

Variation in Spermatozoa.—Zeleny and Senay ('15, Journ. Exp. Zool., 19:505-514) report results of studies on variation in head length of spermatozoa in insects. This work is a continuation of earlier studies by Zeleny and Faust, an abstract of which appears in this journal (34:191). The following insects were studied: *Corisus lateralus, Leptocoris trivittatus, Reduviolus ferus, Euschistus variolarius, Cosmopepla carnifex, Passalus cornutus,* and *Berosus striatus.* With the exception of *Passalus cornutus,* all gave distinctly bimodal curves indicating the existence of two distinct size groups of spermatozoa. The one exception yielded a unimodal curve and is interpreted as indicating lack of distinction among the spermatozoa or else as having two groups which differ so slightly from each other that a unimodal result is produced. Additional support is thus given to the belief that dimorphism in size of spermatozoa is common among animals having two chromosomal classes of spermatids, due to quantitative differences in chromosomal content.

Bibliographies.—The Journal of Animal Behavior (5:407-461) has issued its annual lists of literature pertaining to the behavior of animals. Turner (pp. 415-445) has given a brief analysis of the more important results which have appeared in the literature of 1914 and lists one hundred seven papers from American and foreign sources which treat of the behavior of spiders and insects other

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than ants. The analysis of subject matter is very useful and the list of titles, while not entirely complete, forms an important source of information.

Differential Incidence of Bruchus.—Harris ('15, Journ. N. Y. Ent. Soc., 23:242-253) reports the results of a statistical study on "differential incidence of the beetle Bruchus" in which the purpose was to discover whether the characters of the bean pod, on which the eggs of *Bruchus* are laid, determine, to any degree, the frequency of infestation ("parasitization"). Examination of over fourteen thousand pods, producing almost forty thousand seeds, showed that the relative number of infested seeds is greater in pods with larger numbers of ovules. Increase in percentage of infestation accompanies increase in the number of seeds matured per pod. No relationship between the position of the seed in the pod and liability of infestation was discovered. The hypothesis offered is that since young pod size is correlated with the number of ovules and the number of developing seeds, thus resulting, in the larger pods, increased ease of foothold and additional facility for oviposition are made possible.

Respiration in Zygopterous Larvæ.—Calvert ('15, Ent. News, 26:435-447) summarizes the literature on the subject of the respiration of zygopterous larvæ (Odonata). From compiled and original data it appears that the oxygen demands of the animals are satisfied, at least in part, by several modes of respiration. The general body-surface, the caudal processes, the rectal epithelium, certain spiracles, and, in some species, the lateral external tracheal gills, all form functional parts of the respiratory system.

Behavior of the Ant-lion.—Turner ('15, Biol. Bull., 29:277-307) has studied the behavior of the ant-lion and finds, among numerous other things, that the characteristic pits, which may be found in any kind of dry friable soil in protected situations, are constructed either by furrowing backward, producing a series of concentric excavations, each deeper than the preceding and removing the soil with the head, or by the simple removal of the soil until the sides of the pit become somewhat stable. Locomotion is invariably backward, never forward. "Any invertebrate, be it insect, arachnid, or crustacean, that happens to fall into the trap is accept-

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able as food." Letisimulation (death-feigning) often follows rough handling or other similar treatment and, though variable in duration, mutilation may frequently fail to produce response. No well-defined relation appears to exist between the duration of the feint and temperature, fasting, or strength of the stimulus.

Reaction of Epeira.—Barrows ('15, Biol. Bull., 19:316-326) finds that the large orb weaving spider, *Epeira sclopetaria,* orients itself, when going from the center of the web to capture entangled flies, in response to a vibratory stimulus. The stimulus can be submitted experimentally and produces the normal response, the latter being analyzable into (1) the orientation, (2) the forward movement, and (3) the attack on the vibrating object. Mutilation experiments yielded evidence indicating the probability that the organs used in detecting the vibratory stimulus are sense hairs on the tarsi.

Regeneration.—Schmit-Jensen ('15, Smithsonian Report for 1914, pp. 523-536) made a study of homœotic regeneration of the antennæ in a phasmid, *Carausius morosus.* A "spontaneous case of substitutional homœsis" was noted among a lot of individuals (nymphs) which had suffered from cannibalism. An antenna, bitten off near the base, developed, after molting, a termination consisting of a distinct tarsus-like segment with a large empodium and two small claws. Subsequent molts witnessed the appearance of paired protuberances corresponding exactly to the paired plantulæ on the underside of normal tarsal regions. Amputation experiments on fifty specimens, involving removal of either or both antennæ at the level of the joint between the first and second, or the second and third antennal segments, resulted in the production of twenty cases of regeneration. These regenerations varied to some extent but all developed the tarsus-like terminations. Amputations made on young nymphs showed that regenerations developed more and more with each succeeding molt and the resemblance between the regenerated portion and the true tarsal region became greater. Under certain conditions of the experiments a tibia-like segment was also produced at the base of the tarsal region.

Orientation of Ephemerida.—Krecker ('15, Biol. Bull., 29:381-388) finds that the positive reaction of May-flies to air currents

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is evidently due to strains exerted on the muscles of those appendages which serve the function of attachment, rather than to sensations resulting from contact of moving air with the body. The normal resting position on vertical supports which is usually negative with respect to the earth's surface, is not, apparently, a pure reaction to gravity but is entirely or in part accounted for by the unstable character of the insect's attachment when in any other position. May-flies react negatively to bright sunlight but positively to certain artificial lights. An optimum zone surrounds colorless, sixteen candle-power, incandescent lights, beginning about six inches from each light and extending to about thirty inches, in which the positive reaction seems to be satisfied. Individuals alighting in this zone tend, if large numbers are present, to arrange themselves in rows corresponding to the radii. The area within six inches of the light is designated as the "excitement zone" since individuals entering this zone become excited and perform confused movements. The positive reaction is much stronger for slightly yellowish light than for red or blue. Red and blue lights produce the above-described alignment but a well-defined excitement zone is lacking.

Population of "Blanket-Algæ."—Platt ('15, Am. Nat., 49:752-762), in a study of the life of floating masses of filamentous algæ ("blanket-algæ") in fresh-water pools near Ithaca, N. Y., finds that insects are prominent components of such a community. The larvæ, nymphs, and adults of five orders of insects are regular inhabitants of the alga-masses and constitute the largest individuals of the population. Nymphs of Ephemerida (*Callibætes, Bætis,* et al), nymphs of Odonata (*Enallagma, Ischnura,* et al), larvæ of Diptera (*Chironomus, Ceratopogon, Odontomyia*), larvæ of Coleoptera (*Hydroporus,* et al), and adult beetles (*Helophorus, Crenophilus,* et al) were found to constitute the principal insect population, the Diptera representing the greater part. *Chironomus* was one of the two animal forms which appeared most regularly. Members of this genus were present at all seasons. The larvæ of May-flies and midges are the principal plant consumers and since the

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latter are so prolific they are of great importance as food for other animals. Important data concerning the plant and other animal members of this population are given.

Lepidopterous Larvæ.—Fracker ('15, Illinois Biological Monographs, 2:1-169), in an extensive paper on the classification of lepidopterous larvæ, reports, among others, the following general results: The primary setæ, which constitute important taxonomic characters, have an arrangement in all segments of the body of the larva which has been derived from the same ancestral type, the latter including twelve primary setæ which are arbitrarily designated by Greek letters. Modification of this type has come about in three different and independent ways, namely, tendency of prothorax to retain the maximum number of setæ, partial reduction on the meso-and metathorax, and reduction of abdominal chætotaxy without much change of positions. Other general conclusions of a more special nature are also given. Extensive keys to families, genera and species are presented, accompanied by detailed discussion. This paper covers the whole order Lepidoptera and is the most comprehensive and thoroughgoing treatment of the subject which has yet appeared.

Poisons of Plant-Lice.—Dewitz ('15, Ann. Ent. Soc. Am., 8:343-346) reports that the diluted extract of certain plant-lice obtained by triturating these animals in physiological salt solution or a mixture of glycerine and physiological salt solution, when submitted to ox blood, hæmolyzes the red blood-corpuscles even at ordinary temperatures. "1 gr. of plant-louse matter will completely dissolve the red blood-corpuscles of 25 ccm. of undiluted blood or 40 gr. will dissolve a litre of blood." The substance causing this hæmolysis is given the name *aphidolysin.* Nothing is known concerning the particular part of the body of the insect which contains this poison.

Stimuli and Egg Hatching.—Severin, Severin, and Hartung ('15, Psyche, 22:132-137) have carried on experiments to determine the stimuli which cause the eggs of *Chætogædia monticola,* a leaf-ovipositing tachinid (Diptera), to hatch. These eggs are deposited on various grasses and weeds and, at the time of oviposition, contain fully developed larvæ. Since the larva is a parasite in certain

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other insects which consume the vegetation on which these eggs occur, what accounts for the sudden hatching in the digestive tract of the host which permits the parasite to begin penetrating the wall before being expelled with the excreta? Eggs, placed in the alkaline liquids exuded from the mouths of certain host caterpillars, began hatching within less than one minute, almost all of them hatching within three hours. Similar results were obtained using alkaline liquids from insects which are not the normal hosts. Hatching occurred, in most cases, in the mid-intestine. Emergence was partially prevented, sometimes completely so, in various acid media. Experiments seemed to show that increased turgidity causes the larvæ to emerge when the eggs were immersed in water or diluted alkaline solutions for thirty-six hours or longer. Evidence was secured which points to the probability that the digestive juices of the host, reaching the larva through the micropyle, stimulate it to perform the body movements and contractions by which the escape from the chorion is affected.

Polyembryonic Development.—Patterson ('15, Biol. Bull., 29:333-372) finds that in *Copidosoma gelechiæ,* a hymenopterous parasite of the *Solidago* gall-moth *(Gnorimoschema gallæsolidaginis),* polyembryonic development occurs, the egg giving rise, on the average, to about 191 individuals. The cleavage stages were not secured, the earliest development studied being that in which the division of the egg into embryonic primordia had begun. The young polygerms have an outer nucleated membrane and a central region containing the true embryonic nuclei. The latter, by segregation into groups surrounded by a dense layer of granular protoplasm, develop into the primordia of the multiple embryos. The polygerm elongates, breaks up into several spherical primary masses, each of which contains several primitive embryos. Primary masses give rise to secondary masses and the embryos separate from each other, each developing a covering composed in part of granular protoplasm and in part of protoplasm from the nucleated membrane. The intervening space becomes filled with an inter-embryonal substance, the subsequent dissociation of which liberates the larvæ, thus introducing them into the body-cavity of the host where they ultimately destroy the unchitinized parts of the caterpillar.

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The emergence of males and females from the same caterpillar is interpreted as the result of the deposition of two or more eggs in the same host. However, the possibility "that such broods may also arise from a single fertilized egg by a process of disjunction of the sex chromosomes during the early cleavage stages" is suggested.

Gynandromorph Bees.—Morgan ('16, Am. Nat., 50:37-45), in a paper on the Eugster gynandromorph bees, reviews the evidence pertaining to the two chief theories as to the origin of these anomalous insects. Recent studies by Boveri and Mehling on the original Eugster gynandromorphs show that the male parts of these bees are *maternal* while the female parts are *paternal*, thus supporting the original hypothesis of Boveri that these anomalous insects are produced by a delayed fertilization or by some irregularity in the penetration of the sperm into the egg, resulting in the fusion of the sperm nucleus with one of the two nuclei produced by the division of the egg, with the ultimate production of an individual which posses the characters of the male on one side and those of the female on the other. Morgan's theory of gynandromorph origin is based on the possible entrance of two or more spermatozoa into the egg with a subsequent union of one with the egg nucleus and the independent development of an outlying spermatozoan, the former producing female structures and the latter producing male structures. According to the theory of Boveri, the male parts resulting from the single egg nucleus should be *maternal* while by Morgan's theory the male parts derived from the single sperm nucleus should be *paternal*. Attention is called to the discovery by Newell that the drone bees inherit the characters of the mother. It thus appears that Boveri's theory holds, at least for the case of the honey-bee. Boveri's cytological argument in support of his theory is reviewed but it is found by Morgan to be of uncertain support. Mention is made of a third possible origin for gynandromorphs, namely, "dislocation during ontogeny of the two sex chromosomes." Gynandromorphs would be expected to arise in insects when conditions would prevail in which certain nuclei come to contain two sex chromosomes and others only one. Attention is also called to Goldschmidt's explanation of the gynan-

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dromorphs which he secures in crosses of *Lymantria dispar* and *L. japonica*, an explanation which "involves the relative potencies of the sex factors in the different races."

Marine Insects.—Arndt ('15, Proc. Indiana Acad. Sci., 1914, pp. 323-336) presents a paper on the habits of the insects and spiders of the "between tides zone" at Cold Spring Harbor, Long Island. Particular attention is given to *Megamelus marginatus, Grammonata trivittata, Clubonia* sp., *Bembidium constructum, Heterocerus undatus, Bdelidæ* sp. (?), and *Lycosa communis*. The first three are characteristic of the Spartina grass area, while the last four are common in the outer Juncus area. Almost all insects inhabiting the zone between high and low tide present peculiar protective features, some of which are as follows: (1) Certain unique instincts serve to prevent them from being washed away by the tide, as for example, the tenacious clinging to the blades of grass by those insects which inhabit the Spartina zone, the habit of crawling under the gravel, and the wandering about for food only during sunny days at low tide. (2) The instinct of certain forms to swim to the Fucus thallus during the disturbance of high tide, an instinct which is interpreted as one resulting in protection from aquatic enemies. (3) Marked resistivity of tide zone species to drowning. (4) Structural modifications which facilitate locomotion, enabling the possessors to inhabit an environment in which safety may be dependent upon retreat. Attention is called to the necessity of tide zone insects being air breathers since the terrestrial conditions prevail for one-half of the time. During periods of high tide or submergence they are inactive. "The most striking phenomena is the strictly zonal distribution of the insects of the between tides zone."

Salts Required by Insects.—Loeb ('15, Journ. Biol. Chem., 23:431-434) has raised five generations of the banana fly, each of normal motility, on the following nutritive mixture: grape sugar, 0.5 gm.; cane sugar, 0.5 gm.; ammonium tartrate, 0.1 gm.; citric acid, 0.05 gm.; K_2HPO_4, 0.005 gm.; $MgSO_4$, 0.005 gm.; H_2O, 3 cc. No NaCl or $CaCl_2$, other than that which may appear as impurities in the chemicals used, was necessary. K and PO_4 appeared to be indispensable and SO_4 and Mg must be present. These ex-

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periments show that as highly organized an animal as this fly can be reared on a culture medium as simple as that required for certain micro-organisms. Attention is called to the general assumption that the evolution of the higher animals could have occurred only after the appearance of green plants since the latter serve directly or indirectly as food for the former. Although this is true in general of the present fauna there is the possibility that "an evolution of animals as highly specialized as insects might have taken place independently of the existence of green plants." Attention is also called to the results of certain other experiments which indicate that the nitrite and nitrate bacteria are capable of forming carbohydrates from carbon dioxide, or possibly other atmospheric carbon compounds, independently of light, and other micro-organisms might have the same power. Such micro-organisms might furnish the carbohydrates necessary for development of other micro-organisms requiring sugars for their growth. It thus seems obvious that the evolution of animals as complex as the banana fly might have been possible without the existence of chlorophyll.

Inheritance of Pink Coloration.—Hancock ('16, Ent. News, 27:70-82) has made a study of inheritance of the coloration in the unusual pink form of the katy-did, *Amblycorypha oblongifolia*. He has found that this pink katy-did crosses freely with the normal green form. When a pink female was crossed with a normal green male, some of the hybrid F_1 progeny emerged from the egg after two years while the rest of the eggs hatched after a period of three years. Nine of the hybrids had pink coloration like the mother, and four were green like the male parent. The sexes were about evenly divided in both the pink and the green forms. These hybrid F_1 types have been inbred but the F_2 generation has not yet emerged. The pink and green color which appeared after the first molt was not materially affected by subsequent ecdyses. The pink and the green colors are hereditary and are regarded as germinal in origin. The theory that the color is the result of absorption of coloring matter accompanying the food is rejected.

PAUL S. WELCH.

Kansas State Agricultural College.

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PROCEEDINGS

of the American Microscopical Society

MINUTES OF THE COLUMBUS MEETING

The thirty-fifth annual meeting of the American Microscopical Society was held in connection with the A. A. A. S. at Columbus, Ohio, Dec. 29, 1915.

In the absence of President Kofoid, Professor Henry B. Ward acted as chairman. The meeting was called to order at 5 P. M.

The report of the Custodian was read and referred to an auditing committee, consisting of Dr. H. J. Van Cleave and Professor Frank Smith, both of Urbana, Ill.

On motion of Past-president Bleile the Secretary was instructed to send the greetings of the Society to Mr. Pflaum and to express to him our regret that he was unable to be present.

The Treasurer's report was read and accepted, being referred to the auditing committee named above.

The term of Treasurer Hankinson expired at this meeting, and the Society acquiesced with regret in his decision not to accept the office for another term. Unanimously the members present voiced their appreciation of his faithful and effective service of the Society during six years of its history, including the difficult period of reorganization.

The following officers were duly nominated and elected: President, Professor Michael F. Guyer, University of Wisconsin; First Vice-President, Professor T. L. Hankinson, Eastern Illinois Normal School; Second Vice-President, Professor L. E. Griffin, University of Pittsburg; Treasurer, Dr. Harley T. Van Cleave, University of Illinois. Dr. George R. LaRue, University of Michigan, Professor H. S. Brode, Whitman College, and Professor W. M. Rankin, Princeton University, were elected members of the Executive Committee.

The Secretary was selected as a member of the Council of the American Association for the Advancement of Science and was authorized to name as the second representative for 1916 some member actually in attendance at that meeting.

On the recommendation of the Executive Committee it was voted to allow the Secretary $50.00, or such part thereof as may be necessary, in defraying the expense of attending the annual meetings of the Society.

The Chairman and Secretary were empowered to approve the minutes and prepare them for publication.

The Society adjourned.

T. W. GALLOWAY, Secretary.

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SPENCER-TOLLES FUND

Custodian's Report for the year 1915

Reported at Philadelphia Meeting		$4184.65
Dividends received	$ 254.67	
Life membership No. 6, Seth Bunker Capp	50.00	304.67
Total		$4489.32
Net increase during the year		$ 304.67

GRAND TOTAL

All contributions to date	$ 800.27	
All sales of proceedings	758.38	
All life memberships	300.00	
All interest and dividends	2820.67	$4679.32

LESS

All grants to date	$ 150.00	
All dues on life memberships	40.00	190.00
Balance invested		$4489.32

Life Members: (Robert Brown, dec'd.); J. Stanford Brown; Seth Bunker Capp; Henry B. Duncanson; A. H. Elliott; John Hately.

Contributors of $50 and over: John Aspinwall; Iron City Microscopical Society; Magnus Pflaum; Troy Scientific Society.

MAGNUS PFLAUM, Custodian.

Columbus, Ohio, Dec. 29, 1915.

We, the undersigned committee hereby certify that we have carefully examined the foregoing account of Magnus Pflaum, Custodian, and found the same correct.

H. J. VAN CLEAVE,
FRANK SMITH,
Auditing Committee.

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ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

December 24, 1914, to December 24, 1915

RECEIPTS

Balance on hand from 1914	$ 243.61
Dues of old members	442.87
Dues of new members	70.00
Initiation fees	102.00
Subscriptions for volume 33	100.00
Subscriptions for volume 34	34.40
Subscriptions for volume 35	26.00
Subscriptions for other volumes	103.00
Sales of Transactions	3.30
From Y. H. Tsou, as part expense of publishing his paper	75.00
From H. E. Metcalf, as part expense of publishing his paper	25.00
Life membership of S. B. Capp	50.00
Advertisers in volume 33	131.25
Advertisers in volume 34	18.50
Advertisers in volume 35	7.50
Advertisers in other volumes	90.00
Total	$1,522.43

EXPENDITURES

Printing Transactions, volume 33, no. 4	$ 198.29
Printing Transactions, volume 34, no. 1, 2, 3	483.65
Plates for Transactions, volume 33, no. 4	6.25
Plates for Transactions, volume 34, no. 1, 2, 3	37.70
Plates for volume 34 no. 4	24.22
Postage and express for Secretary	41.61
Postage and express for Treasurer	18.50
Office expenses, stationery, stenography, etc.	126.40
Office expenses of the Treasurer	9.10
Advertising literature	39.88
Spencer-Tolles fund from S. B. Capp's dues	50.00
Sundry	10.18
Balance on hand	476.65
Total credits	$1,522.43

T. L. HANKINSON, Treasurer.

Signed by Auditing Committee,

We hereby certify that we have examined this account, have checked items against vouchers on file, and have verified the totals. We find the report as given above to be correct.

H. J. VAN CLEAVE,
FRANK SMITH,
Auditing Committee.

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ORGANIZED 1878 INCORPORATED 1891

APRIL
1916

VOLUME XXXV No. 2

TRANSACTIONS
OF THE
AMERICAN MICROSCOPICAL SOCIETY

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Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants
from the Spencer-Toller Research Fund.
(See TRANSACTIONS, July, 1913)

These awards are to stimulate and aid research in Microscopic work

1. **The Grantee must be a member of the Society**
2. **Resulting discoveries must first be offered for publication in the Society Transactions.**
3. **Application should be made to Dr. Henry B. Ward, Chm., Urbana. Ill.**

NOTICE TO MEMBERS

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the Transactions.

T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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TRANSACTIONS

OF THE

American Microscopical Society

ORGANIZED 1878 INCORPORATED 1891

PUBLISHED QUARTERLY

BY THE SOCIETY

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R. H. Ward, M.D., F.R.M.S., of Troy, N. Y.,
at Indianapolis, Ind., 1878, and at Buffalo, N. Y., 1879

Albert McCalla, Ph.D., of Chicago, Ill.
at Chicago, Ill., 1883

Geo. E. Fell, M.D., F.R.M.S., of Buffalo, N. Y.,
at Detroit, Mich., 1890

Simon Henry Gage, B.S., of Ithaca, N. Y.,
at Ithaca, N. Y., 1895 and 1906

A. Clifford Mercer, M.D., F.R.M.S., of Syracuse, N. Y.,
at Pittsburg, Pa., 1896

A. M. Bleile, M.D., of Columbus, Ohio,
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C. H. Eigenmann, Ph.D., of Bloomington, Ind.,
at Denver, Colo., 1901

E. A. Birge, LL.D., of Madison, Wis.
at Winona Lake, Ind., 1903

Henry B. Ward, A.M., Ph.D., of Urbana, Ill.,
at Sandusky, Ohio, 1905

Herbert Osborn, M.S., of Columbus, Ohio,
at Minneapolis, Minn., 1910

A. E. Hertzler, M.D., of Kansas City, Mo.,
at Washington, D. C., 1911

F. D. Heald, Ph.D., of Philadelphia, Pa.,
at Cleveland, Ohio, 1912

Charles Brookover, Ph. D., of Little Rock, Ark.,
at Philadelphia, Pa., 1914

Charles A. Kofoid, Ph.D., of Berkeley, Calif.,
at Columbus, Ohio, 1915

The Society does not hold itself responsible for the opinions expressed by members in its published *Transactions* unless endorsed by special vote.

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(This Number was issued on May 25, 1916.)

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Installments)

Vol. XXXV APRIL, 1916 No 2

GRANTS FROM THE SPENCER-TOLLES FUND

By T. W. GALLOWAY, *Secretary*

The Spencer-Tolles fund is an endowment to encourage research. It has been accumulated by the American Microscopical Society thru a period of thirty-two years by means of Life Memberships, by gifts of individuals and societies, by sale of Transactions, and by accumulated interest. It is intended as a memorial to C. A. and H. R. Spencer and R. B. Tolles, pioneer workers in America in optical instruments. It has been the desire to have this memorial a permanently active one,—continually stimulating scientific work and lending to scientific progress in the field so long served by the American Microscopical Society.

The funds are favorably invested and will soon amount to $5000. Until that sum is reached it is the purpose of the committee to award less than the annual income each year. In the course of two or three years it is the hope that the annual grants may total $250 or $300.

Members of the American Microscopical Society are invited to aid the Society in reaching the ends for which the fund was established. In aiding the Committee find the very best avenues for the awarding of the grants, members will benefit themselves and the membership at large, since the results of the researches will be published in the *Transactions*. It is the confident expectation that the Spencer-Tolles Fund will not only advance research, but greatly strengthen the American Microscopical Society during the years to come.

The terms regulating the making of grants from the income of the fund, quoted from the announcement of the Committee, are given herewith.

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Regulations Governing Grants from the Spencer-Tolles Fund.

1. The Committee will receive formal application for grants from the Spencer-Tolles Fund at present only from members of the American Microscopical Society.

2. For the present, under ordinary circumstances not more than $100 will be voted in any one year to research purposes. No money can be granted for any other purpose from the income of this Fund.

3. Applications for grants shall be filed with the chairman of the committee.

4. On completion of the work each recipient of a grant shall give a report of the use to which the grant allowed has been put, preferably in the form of a paper ready for publication and embodying the results of the work in connection with which the grant was used.

5. Every grant is made upon the express condition that all results obtained by its aid shall be offered to the American Microscopical Society for publication in advance of their announcement elsewhere. Wherever published, the publications including the results of this work shall contain the distinct statement that the work contained in the paper was done with the aid of a grant from the Spencer-Tolles Fund of the American Microscopical Society.

6. The expenditure of the money shall be entirely in the control of the person receiving the grant and he shall not be asked to secure or furnish any vouchers covering the expenditure in detail. On completion of the work he shall file with his report a statement that such a sum, mentioning the amount, has been expended and the results of the work are contained in the accompanying report. Any unexpended balance retained by the custodian in making the final payment, or if paid out of the grant not covered by this statement, shall be returned to him and shall be again placed in the Fund.

(Signed) HENRY B. WARD, Chairman,
S. H. GAGE,
MAGNUS PFLAUM,
H. R. HOWLAND,
A. M. BLEILE.

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The Committee or the Secretary will welcome correspondence from members regarding research work which for its preparation or publication needs the assistance of this fund. It is the purpose to interpret the conditions with the utmost generosity in the interest of scientific discovery. It is not the desire to hamper the investigator by limiting the use of grants to very precise purposes or control narrowly their expenditure. The fullest possible freedom for the exercise of individual judgment will be allowed the grantee.

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SNOW-FIELD AND GLACIER OLIGOCHÆTA FROM MT. RAINIER, WASHINGTON*

By Paul S. Welch

The material which forms the basis of this paper was collected on the snow-fields and glaciers of Mt. Rainier, Washington, during February, March, April, and June, 1915. Specimens first came to the writer through the courtesy of Professor Frank Smith, of the University of Illinois, to whom they had been sent by the United States Bureau of Biological Survey. The collections were made by Mr. J. B. Flett of the Longmire Ranger Station who later supplied the writer with six collections from the same region. The writer wishes to express his indebtedness to Mr. Flett for his continued interest and his many courtesies in furnishing carefully preserved material and in supplying data on the habits and appearance of the living worms.

Genus Mesenchytræus

Both of the species discussed in this paper belong to the genus *Mesenchytræus (Enchytræidæ)*, which, at the present time, includes almost sixty species and varieties, a few of which are of uncertain standing. Of this assemblage, twenty species and two varieties are recorded from North America. They are as follows, the type locality for each being given: *beringensis* Eisen (Bering Island, Bering Strait, Alaska), *beumeri* Mchlsn. (?) (Hamburg, Germany), *eastwoodi* Eisen (Hoods Peak, Sonoma Co., Calif.), *fontinalis* Eisen (Pine Ridge, Fresno Co., Calif.), *fontinalis* var. *gracilis* Eisen (Fresno Co., Calif.), *franciscanus* Eisen (San Francisco, Calif.), *fuscus* Eisen (Pit River, Calif.; No. Calif.), *fuscus* var. *inermis* Eisen (West Fork of Feather River and Goose Lake, Modoc Co., Calif.), *grandis* Eisen [Alaska (Sitka? or Juneau?)], *harrimani* Eisen (Kadiak, Orca, Metlakatla, Sitka, Yakutat, Unalaska, Alaska; Lowe Inlet, British Columbia), *kincaidi* Eisen (Ice-House Lake, St. Paul Island, Bering Sea, Alaska), *maculatus* Eisen (Popof

*Contribution from the Entomological Laboratory, Kansas State Agricultural College, No. 18.

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Island, Alaska), *nanus* Eisen (Popof Island, Alaska), *niveus* Moore (Mt. St. Elias, Alaska), *obscurus* Eisen (St. Paul Island, Pribilof Group; Popof Island, Alaska), *orcæ* Eisen (Orca, Alaska), *pedatus* Eisen (Goose Lake, Alturas, Modoc Co., Calif.), *penicillus* Eisen (Port Clarence, Alaska), *setchelli* Eisen (Unalaska Island, Alaska), *solifugus* Emery (Muir Glacier; La Perouse Glacier, Alaska), *unalaskæ* Eisen (Unalaska, Alaska), and *vegæ* Eisen (Port Clarence, Alaska).

All but one of the above-mentioned species were originally described from North America and are not yet known to occur elsewhere. *Mes. beumeri* Mchlsn., a European species, has been doubtfully reported by Moore ('99, p. 141) from the vicinity of Philadelphia, Pa. He also reported an undescribed species of this genus from the same locality. The last American contribution to the knowledge of *Mesenchytræus* is that of Eisen ('05) in which most of the above-listed species are described. However, since that time new representatives of the genus have been reported from other parts of the world.

Mesenchytræus gelidus n. sp.*

(Plates XIV-XVI; Figs. 1-19)

Definition.—Length of alcoholic specimens, 21-32 mm., average about 24.7 mm. Diameter, 1.25 mm. Somites, 66-77. Color, dark reddish brown to almost black. Prostomium blunt, rounded, smooth. Head pore at tip of prostomium. Setæ sigmoid; all of same size and approximately uniform in length; in anterior part of body, 4-5 in lateral bundles, and 7-9 in ventral bundles; in posterior part of body, 3-4 in lateral bundles and 4-6 in ventral bundles. Clitellum on ¾XI-XIII, continuous around body. Two septal glands on IV/V and V/VI. Brain with width two to two and one-third times the length; anterior margin deeply emarginate, posterior margin almost straight, lateral margins diverging cephalad. Dorsal blood-vessel arises in XIV; cardiac body present. Nephridia with small, slender anteseptal part and large, irregular, compressed postseptal part; efferent duct arises from ventral surface

*A typographical error which the writer was unable to correct appears in the abstract of this paper (Welch, '16, p. 143). The name of this species appears therein as *gelicus* instead of *gelidus*.

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of latter about mid-way of its length. Spermiducal funnel large, somewhat cylindrical, bent; length three to four times the diameter; collar present, variable, usually reflected or flaring, set off from body of funnel by distinct, broad constriction. Sperm duct 6-7 times length of funnel. Penial bulb large, somewhat globular; atrium globular but smaller than body of bulb; about twelve, elongate, finger-like, multicellular glands opening into ental extremity of atrium about entrance of sperm duct; two sets of glands within penial bulb. Sperm sacs extend caudad to XXXI–XXXV. Ovisac single, tubular, bifurcates in XVI; extends to XXXI–XXXV; contains sperm sacs. One pair of unusually developed spermathecæ; ectal opening laterad at IV/V, two inconspicuous groups of unicellular glands, surrounded externally by definite, light yellowish area; duct short, cylindrical, symmetrical, straight; two well-developed diverticula at ental end of duct, oppositely placed, elongate, slightly expanded at extremities; ampulla very long, usually terminating in IX–XI; devoid of connection with digestive tract; irregular, more or less unsymmetrical, constricted in regions corresponding to position of septa, caudal end usually dilated.

This description is based on twenty-seven sexually mature specimens. Many others of uncertain sexual maturity were examined in the study of external characters. The type and most of the paratypes are in the collection of the writer. Paratypes have also been deposited in the collection of the United States National Museum and in the collection of Professor Frank Smith.

The habitat of this species will be described in some detail in another part of the paper.

Affinities.—The determination of the affinities of this enchytræid is a matter of considerable difficulty, especially when the foreign species are considered. Many of the latter, recorded a number of years ago, are not described in sufficient detail to make possible a profitable attempt to discover relationships. Furthermore, the assemblage of species assigned to *Mesenchytræus* includes a number of poorly described ones, such as *Mes. armatus* Lev., *Mes. mencli* Vejd., and *Mes. montanus* Bret., the affinities of which cannot be judged either because of the failure to describe

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a number of the essential details of structure or because of the use of sexually immature specimens. The validity of several foreign species seems to be questionable but, until they and other poorly described forms receive more intensive work, little can be done in the accurate determination of the synonymy. After a careful scrutiny of the literature dealing with the foreign species, the writer has not found any of them, as described, to closely approach *Mes. gelidus.*

Mes. gelidus belongs to the group of species having two diverticula on each spermatheca. This single point of agreement does not necessarily indicate close relationship and it is very possible that the convenient grouping of species on the basis of the number of diverticula is somewhat artificial. However, *Mes. gelidus* belongs to the group in which the spermathecæ are prolonged caudad through several somites and lack connection with the lumen of the digestive tract. It appears that at least five American species, namely, *Mes. harrimani* Eisen, *Mes. setchelli* Eisen, *Mes. franciscanus* Eisen, *Mes. obscurus* Eisen, and *Mes. maculatus* Eisen, are to be regarded as close relatives. They have been described in considerable detail and fairly satisfactory comparisons are possible.

Mes. harrimani differs from *Mes. gelidus* in having a length of more than twice the average of the latter; a larger number of somites; a smaller number of setæ in both lateral and ventral sets; brain square, not markedly wider than long; a longer and more slender spermiducal funnel; a much shorter sperm duct; and less compact nephridia, each possessing a distinct bladder-like chamber near the ectal opening of the efferent duct. Slight differences occur in the position of the clitellum and in the distribution of pigment. The penial bulb and associated structures are similar in some respects but a satisfactory comparison is prevented owing to a discrepancy in Eisen's description ('05, pp. 24-25) in which the following statement is made: "Atrium medium size, with about sixteen large gland-fascicles opening at the entrance of the atrium into the bulb." In the "Synopsis of species of Mesenchytræus" in the same paper (pp. 18-20) the following statement is made: "Penial glands, about 12 long atrial glands". Eisen's text figure No. 6 shows nine atrial

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glands and another figure (Plate II, Fig. 4) shows fourteen. Both figures are, however, diagrammatic. No discussion of such variation occurs in the description and the writer is at a loss to know what interpretation to put upon the matter. Disregarding the atrial glands, the structure of the penial bulb, particularly the internal glands, differs from that of *gelidus*.

Mes. setchelli differs from *Mes. gelidus* in possessing a rounded brain with a decidedly convex posterior margin; in having the origin of the dorsal blood-vessel in XVIII; in having sperm sacs which reach only to XVIII; and in possessing only five atrial glands in connection with the penial bulb. Smaller differences exist in the character of the spermiducal funnel, nephridia, and in the details of structure of the penial bulb.

Mes. franciscanus differs from *Mes. gelidus* in the distinctly smaller number of setæ per bundle in both sets; in the origin of the dorsal blood-vessel in XVI; in the much longer and more slender spermathecal diverticula; and in the spermiducal apparatus which has a single, large, well-defined accessory gland in connection with the penial bulb, a sperm duct not longer than one and one-half times the length of the funnel, and distinctly sessile, globular, atrial glands. Minor differences exist in the position of the clitellum and in the finer structure of the penial bulb.

Mes. obscurus is distinguished from *Mes. gelidus* by the position of the clitellum on XII and XIII; the very long, slender, spermathecal diverticula; the very slender spermiducal funnel; the loosely constructed, three-lobed nephridia; and the possession of 16-20 atrial glands in connection with the penial bulb.

Mes. maculatus seems to be a close relative of *Mes. gelidus* but differs from it in the number of setæ per bundle in the lateral set; in the deltoid shape of the brain; in the spermathecæ in which the slender diverticula are not located at the junction of the duct with the ampulla and are much shorter than the former; and in the possession of three sets of internal penial bulb glands, one set consisting of multicellular glands.

External Characters

The body of *Mes. gelidus* is elongate, sub-cylindrical, smooth, and of about uniform diameter, except in the regions cephalad of

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the clitellum and near the posterior end where there is a gradual tapering towards the extremities. In alcoholic specimens, there is a slight but distinct dorso-ventral flattening accompanied by a faint, shallow, mid-ventral, longitudinal depression which is present throughout the greater part of the body. However, since the writer has not had the opportunity of studying living material, no statement can be made concerning the constancy of these characters and there is the possibility that they are unnatural results incident to preservation and that the true form is a cylindrical one as is the case in many of the *Enchytræidæ*. The length, in preserved material, varies from 21 to 32 mm., the average of twenty-five sexually mature specimens being 24.7 mm. The maximum diameter, in the region of the clitellum, is about 1.25 mm. The segmentation is distinct in all parts of the body. The intersegmental grooves are narrow, shallow, and regular in outline, except in the vicinity of the extremities where they are broader, deeper, and more conspicuous, particularly on the cephalic end. The deepening of the intersegmental grooves in the regions of the extremities produces an antero-posterior convexity of the surface of intervening somites which elsewhere is uniformly plane. On the surface of each somite, midway between the margins, is a faint elevation or ridge which encircles the body, including the four setæ bundles. A number of the specimens show, in the middle region, a series of ruptures in the intersegmental grooves, thus producing whitish rings which consist of interruptions, more or less regular, of the cuticula and hypodermis, exposing the underlying muscle layers of the body-wall. Further discussion of these ruptures occurs in another part of the paper.

The number of somites varies from 66 to 77, the average of twenty-five specimens being 69. A few mature specimens were studied in which the number was as low as 50 but these were disregarded since there was some evidence of loss of somites and subsequent regeneration in the posterior region. The color of most mature specimens is, in general, deep reddish-brown to almost jet-black. The distribution of color is not uniform. The ventral surface is often of a slightly lighter hue and the anterior and posterior regions are invariably lighter in color. In occasional specimens,

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the terminal somites are light yellow. Sometimes the black color predominates over almost the entire body. A few specimens were found in which a distinct, rather abrupt transition from also black to yellow occurred in the vicinity of LX but they are referred to above as displaying some evidence of regeneration, a possible explanation for the difference in color. The maximum intensity of the black color occurs dorsad and laterad, in the region behind the clitellum. An examination of the surface under magnification shows the universal presence over the body, save on the clitellum, of innumerable, minute, irregularly distributed, light yellow spots, which give the surface a flecked appearance. These microscopic areas are slit-like, the long axes predominantly extending in the direction of the circumference.

Certain, special, superficial areas show distinct color differences. The ectal opening of each spermatheca at IV/V is surrounded by a broadly fusiform area which occupies about one-half the width of the two adjacent somites and is rendered rather conspicuous by the contrast with the surrounding surface. The crescent-shaped mouth and the transverse slit-like openings of the penial bulbs and oviducts are surrounded by narrow but distinct, light yellow areas. On the ventral surface, beginning with VII, a pair of small, circular, widely separated, yellow spots occur on either side of the mid-ventral line and in close proximity to the cephalic margin of each somite. These are the ectal openings of the nephridia.

The clitellum occurs on ¾XI-XIII. Some slight variation in extent was noted but on completely mature specimens the above-mentioned limits hold. It is moderately thick, increasing, to a limited extent, the diameter of the body in that region. The distinct, uniform, yellow color renders it a conspicuous external character. It completely surrounds the body, no diminuation of thickness occurring on the ventral side. The surface is smooth and lacks the flecked appearance described for other regions of the body.

The head pore is distinct externally and located on the apex of the prostomium. The ectal opening has the form of a trans-

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verse slit and is surrounded by a narrow, yellowish area. Dorsal pores are absent.

The setæ are distinctly sigmoid and arranged in fan-shaped bundles which are disposed in four longitudinal rows, two ventral and two lateral. The setæ of a bundle are all of approximately equal development. In the anterior part of the body, there are 4-5 setæ in the lateral bundles and 7-9 setæ in the ventral bundles. In the posterior part, there are 3-4 setæ in the lateral bundles and 4-6 in the ventral bundles. The setæ on XI are not specialized.

Internal Characters

Lymphocytes.—In the alcoholic specimens examined, the lymphocytes (Pl. XIV, Fig. 4) are scanty in the anterior region of the body but posterior to the clitellum they occur in some abundance. They vary in shape to some extent but, in general, are oval or elliptical. All lymphocytes are so heavily loaded with dark, non-staining pigment-granules that the cytoplasm is almost entirely obscured and frequently the nuclei are almost completely hidden.

Chloragog Cells.—Aside from the anterior five or six somites, the digestive tract is covered with chloragog cells (Pl. XIV, Fig. 5) for the greater part of its length. They are closely set together, usually elongated, and expanded at the free ends. In most of the specimens examined, these cells were heavily loaded with numerous, minute pigment-granules. In many instances, the amount of pigment present was almost sufficient to render the ectal portion of the cell unstainable.

Brain.—The brain (Pl. XV, Fig. 10) lies almost entirely in I, although in some of the specimens it extends slightly into II. It is easily dissected out *in toto,* thus facilitating the study of the organ as a whole. In all of the preparations, the shape and proportions are quite constant. The width is approximately from two to two and one-third times the length, an average measurement being: length, 0.132 mm.; width, 0.301 mm.; maximum thickness, about 0.011 mm. The anterior margin is deeply emarginate and slightly angular in character while the posterior margin is very slightly concave. From the rounded latero-caudal angles, the lateral margins diverge cephalad. Two pairs of supporting strands extend to the

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body-wall, one arising from the anterior part near the emargination and the other from the posterior margin.

Nephridia.—The nephridia (Pl. XV, Figs. 14, 18) begin on VI/VII. Their shape varies somewhat in different regions of the body and in different specimens but, in general, the anteseptal part is composed of a nephrostome borne on an elongated, narrow pedicel. The postseptal part is an enlarged, irregular, compressed mass, the posterior end of which is reflected cephalad and gives rise to the efferent duct. In the large majority of the specimens examined, the nephridia are distinctly compressed and lie flat against the ental surface of the body-wall. Structurally, the body of the nephridium is of the usual mesenchytræid type. No evidence of a reservoir at the ectal end of the efferent duct was observed.

Spermiducal Funnel.—This organ (Pl. XV, Figs. 15-17) lies in the usual position in XI. The dimensions show limited variation but the length is commonly from three to four times the maximum diameter. A well-developed, variable collar is present. In all of the specimens examined, the funnels are bent in varying degrees, and the opening is directed caudad. The sperm duct is approximately 6-7 times longer than the funnel and extends caudad, usually to XIII/XIV. It is then reflected cephalad, extending into XII to unite with the penial bulb.

Sperm Sacs and Ovisac.—A large part of the cœlom posterior to the clitellum is occupied by the extensive sperm sacs (Pl. XIV, Figs. 1-2) and the single ovisac. They lie ventrad and laterad of the alimentary canal and are the most conspicuous of the internal organs. They are formed by very long caudal extensions of certain septa in the clitellar region. In the specimens examined, the delicate nature of these septa and the crowded condition of the internal organs in the clitellar region make the exact determination of the origin of the sperm sacs and the ovisac difficult and it is only by careful reconstructions of serial sections that the beginnings of these storage sacs can be followed. Septum XII/XIII continues caudad as a single, tubular outgrowth to XVI where it divides into similar halves, a right and a left, which lie on either side of the median line. The posterior termination, in the specimens exam-

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ined, varies from XXXI to XXXV inclusive. Definite constrictions occur at intersegmental regions while between the latter distinct swellings are present. In sexually mature specimens, developing masses of ova occur in great quantities throughout the entire length of the sac.

Septum XI/XII continues caudad as two tubular outgrowths, one corresponding to each spermiducal funnel, which enter the ovisac and are completely contained within the latter and its branches. In XVI, where the ovisac divides, each sperm sac enters the corresponding branch of the ovisac and is coterminal with it, the variation in the position of the end being XXXI-XXXV. The sperm sacs also show constrictions and swellings which correspond to the segmentation. They fill approximately one-half of the lumen of the ovisac and, in sexually mature specimens, are crowded full of developing spermatozoa. The opening of each spermiducal funnel is directed towards, and is in close proximity to, the anterior opening of its corresponding sperm sac, in some specimens being partly contained within it. The sperm duct apparently lies entirely outside of these sacs.

A somewhat similar extensive provision for the storage of the developing reproductive cells has been reported previously in a few other species. Eisen ('05, pp. 25, 47, 49) found that in *Mes. harrimani* the sperm sacs extend "back some thirty somites" but made no mention of an ovisac; that in *Mes. fuscus* the sperm sacs are very large, "extending as far back as somite XXVII or further"; and that in *Mes. fuscus* var. *inermis* the sperm sacs extend to XXII and the ovisac to XXVIII. Other species are described in which sperm sacs and ovisacs are recorded as "extending far back" or by some other similar, indefinite statement. Many descriptions contain no mention of sperm sacs or ovisacs. Until such described species have been re-examined, it will not be possible to know whether or not these extensive sacs are peculiar to the American fauna.

Penial Bulb.—In general structure, the penial bulbs (Pl. XVI, Fig. 19) conform to the mesenchytræid type of Eisen. They present a noteworthy complexity of structure and agree in a number of respects with the same organs in certain Pacific Coast species.

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They are attached to the ventral, ental surfaces of the body-wall in XII, one on either side of the median line. Each organ occupies a large part of the cœlom, having a total length, exclusive of the atrial glands, of about two-thirds the diameter of the body in that region. The maximum diameter slightly exceeds one-fourth of the transverse body-dimension. In the specimens studied, it was easy to dissect out these organs and to study them *in toto* as well as in serial sections.

Each organ is composed of two distinctly differentiated regions: (1) the globular atrium, and (2) the slightly elliptical body of the bulb. Superficially, the sperm duct unites with the ental apex of the atrium and is uniform in diameter from that point to the spermiducal funnel. About twelve, well-developed, finger-like, atrial glands are attached to the apex of the atrium near the union with the sperm duct. All are similar in shape and dimensions and extend freely into the cœlom. The attachments of these glands are not distributed uniformly about the apex of the atrium but are aggregated largely on one side.

The internal structure of the penial bulb is complicated and presents some interesting detail. In the completely retracted condition, the extension of the sperm duct forms at least one-half of the length of the bulb as a whole. The remainder of the organ is that part surrounding the penial bulb invagination. The continuation of the lumen of the sperm duct widens within the atrium to form a definite, spacious chamber, corresponding in contour to the external surface of the globular atrium. This chamber, at its ectal side, leads into an elongated lumen which expands slightly, forming another chamber, just entad of the penial pore. This expansion seems to correspond to the "penial chamber" described by Eisen ('05, p. 8). The penial bulb invagination is deep, bounded on the mesal side by a rather smooth wall, but on the opposite side two strong folds are present. It is lined throughout by a continuation of the external cuticula. The lining epithelium of the atrium differs from the corresponding tissue in the sperm duct in that the cells are elongated, reduced in transverse dimension, and lack cilia. The muscle-layer is in close proximity to the bases of these cells and while it varies somewhat in thickness and is interrupted at

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intervals, it can be traced to its origin from the circular muscle-layer of the body-wall. The epithelium, at the penial pore, gradates into the hypodermis which forms the greater part of the lining of the penial bulb invagination. Structurally, it differs from the peripheral hypodermis only in the reduced length of the cells and the absence of the heavy, ectal zone of pigment, except at the entrance of the invagination and on the extremity of the first fold. The lateral portion of the bulb contains a large number of loosely associated muscle-strands which extend, in general, from the periphery towards the interior. The retractor muscle is formed largely by the union of strands from the circular muscle-layer of the body-wall with two other bands, each extending into one of the large folds which project into the invagination. The mesal side of the bulb, particularly near its base, also contains a loose meshwork of muscle-strands. In the ental half of the organ, a circular muscle-layer is present, exterior to and in contact with the longitudinal muscle-layer. The former is particularly well developed in the lateral half of the bulb. It seems probable that this circular layer is a continuation of the longitudinal muscle-layer of the body-wall. A very delicate peritoneum separates the muscle-layers from the cœlom.

Sections show that the atrial glands are composed of two very definite regions, a peripheral region and a central one. The peripheral region is constructed of large, somewhat cuboidal, distinctly nucleated, gland cells arranged in a single layer. The central region is composed of the extensions of the peripheral gland cells which evidently function as ducts. The gland cells take artificial stains intensively but the central region seems to have little or no affinity for them. These extensions of the gland cells composing the central region penetrate the wall of the atrium; extend beneath the muscle-layers, separating the latter from the epidermal lining of the atrial and "penial" chambers; and open into the penial bulb invagination on the surface adjacent to the penial pore.

Two sets of glands occur within the penial bulb. Many large unicellular glands of varying size and shape intermingle with the strands of the circular muscle-layer in the ental half of the organ. It has not been possible to follow out the extensions of these cells

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and determine with what part of the lumen they are related. Another set of glands is present in the lateral side of the ectal half of the organ and are related to the penial bulb invagination. Small unicellular, fusiform, gland cells occur in the heavy folds of the wall, in close proximity to the hypodermis. Each gland has a very fine extension which is related to the hypodermis but the exact nature of this relation has not been determined. It seems probable that these prolongations extend to the surface of the cuticula.

Spermathecæ.—Among the prominent internal organs of the body is a pair of spermathecæ (Pl. XV, Figs. 7-8, 12-13) which first appears in V. These organs are greatly elongated, often extending caudad as far as XI and occupying the large part of the cœlom in that region. Each organ is composed of three distinctly differentiated parts, namely, duct, diverticula, and ampulla. The duct is straight, elongate, cylindrical, and slightly greater in diameter near its middle. The ectal opening is laterad in position in IV/V, slit-like, and surrounded on the external surface of the body by the distinctly light colored area already described. Two groups of unicellular glands are associated with the ectal opening, one on the cephalic and the other on the caudal side. The component glands of each group are elongate, club-shaped, and distinctly nucleated. These groups of glands are apparent only in sections and because of their small size may be overlooked in a casual examination. At the junction of the duct with the ampulla are two, smooth, club-shaped, oppositely situated diverticula, each of which is slightly longer than the spermathecal duct and is reflected caudad, parallel with the long axis of the ampulla. The bulk of the spermatheca is composed of the greatly elongated ampulla. It is characterized by a series of swellings and constrictions, the latter corresponding to the intersegmental grooves, and the whole ampulla often has a moniliform appearance. There is no connection of any kind with the digestive tract but the caudal, free extremity of the organ is an expanded sac, variable in form, and usually larger than any of the other distended portions of the ampulla.

The spermatheca shows some interesting variations. The duct and diverticula are comparatively constant in shape and size but the ampulla is variable. Commonly, the ampullæ in the same speci-

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men are of equal development and approximately of the same shape but a number of specimens were examined in which one ampulla (Pl. XV, Figs. 12-13) was greatly elongated, extending into IX, while the corresponding one on the opposite side of the animal was reduced, extending through only one or two somites, and considerably different in shape. The usual position of the ampulla is parallel to and latero-ventral of the digestive tract but specimens were examined in which the ampullæ were somewhat contorted and partially wrapped around the digestive tract. It is possible that such a condition is responsible for the reduced size of one ampulla since in certain specimens the ampulla on one side had apparently exceeded the other in growth, had filled the available space on its side of the cœlom, and had become crowded to the opposite side while the ampulla of the opposite spermatheca had grown caudad until it came into contact with the other one and had evidently ceased development, resulting in its reduction in length. An examination of these reduced ampullæ *in situ* sometimes showed the free extremities in contact with the opposite ampullæ and doubled upon themselves, suggesting a crowding of parts in development.

The excellent state of preservation of the specimens made possible a study of the finer structure of the spermatheca. The external cuticula is reflected into the ectal opening and forms a complete lining for the lumen of the spermathecal duct, disappearing at the bases of the diverticula. The hypodermis merges into the lining epithelium of the duct which composes by far the greater part of the thickness of the wall. The cells are elongated, glandular in appearance, closely set together, and distinctly nucleated at their bases. They are longer in the middle region of the duct and are responsible for that position of the maximum diameter of the organ. At the ental end of the duct, these cells gradate rather abruptly into the lining epithelium of the diverticula. The circular muscle-layer of the body-wall forms the longitudinal muscle-layer of the duct for its entire length, diminishing in thickness at the bases of the diverticula. It has not been possible to determine whether the longitudinal muscle-layer of the body-wall is related to the spermatheca. Unicellular glands occur over the outer surface of the duct throughout its entire length.

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The walls of the diverticula are composed of a lining of epithelium, constructed of cuboidal, closely set, glandular, distinctly nucleated cells, bounded on their ectal ends by a very thin muscle-layer which, in turn, is covered by a continuation of the above-mentioned peritoneum.

Structurally, the ampulla is composed of two regions, a short ectal portion adjoining the duct, and a very long ental part extending caudad. The lining epithelium of the ectal region shows numerous transverse folds, thus increasing its surface to a considerable extent. This epithelium usually has irregularly shaped, small, non-staining pigment-granules in the free ends of the cells. The muscle-layer is well developed in this part of the diverticula. Unicellular glands occur exterior to the muscle-layer and are bounded at their outer ends by the peritoneum. In the remainder of the ampulla, the wall is greatly reduced in thickness so that the layers are difficult to distinguish. A thin lining epithelium and a delicate peritoneum are present and there are some hints of the presence of a muscle-layer, the exact character of which could not be determined. Small, scattering, irregular thickenings occur on the inner surface of this region.

The contents of the spermathecæ present some interesting features. In all of the sexually mature specimens examined, the spermathecæ contain spermatozoa which have a definite distribution in the organ. No spermatozoa were found in the duct but the diverticula almost invariably contained them, each mass having a definite and constant relation to the epithelial lining. The heads are all in contact with the lining of the diverticulum and the tails extend out into the lumen. The meaning of this arrangement is not clear. Spermatozoa are almost constantly absent from the ectal portions of the ampulla but, in the long, expanded part, surprising quantities, crowded into large masses, are present in the lumen and are not related to the wall in any way.

The striking structural feature of the spermathecæ is the unusual size. It appears from a study of the literature on *Mesenchytræus* that greatly enlarged and elongated spermathecæ, such as the type just described, have been found only in American species from the Pacific Coast. Eisen ('05) described such organs for the

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first time in the following species: *Mes. harrimani, Mes. setchelli, Mes. franciscanus, Mes. obscurus, Mes. maculatus, Mes. vegæ,* and *Mes. orcæ.* As descriptions stand at present, it appears that in this large genus only a small group of species possesses this particular type of spermatheca. Eisen ('05, p. 15) makes the following statement in this connection: "There is some little reason to suspect that this enlargement of the spermathecæ in this genus may have been overlooked in some species, and that some spermathecæ which have been described as short and as immediately connecting with the intestine, in reality are greatly prolonged posteriorly. The part adjoining the diverticles is always narrow and closely approaches the intestine. This peculiarity causes it to tear readily and I am satisfied that such torn spermathecæ have been considered as entire." Whether Eisen's speculations are true remains to be proved but since some of the descriptions of foreign species are very meager, unsatisfactory, and evidently made without careful dissection of specimens or the study of serial sections, it is possible that this type of spermatheca is not quite as unique as it now seems. However, since the spermathecæ have long been used as a taxonomic character and therefore called for special attention, it would appear that the number of cases in which such exceptionally large organs would be overlooked must, at best, be quite small. An inspection of the above list of species possessing such spermathecæ shows that these forms are all Alaskan in distribution, save *Mes. franciscanus.* It might be suspected that greatly enlarged and prolonged spermathecæ are characteristic of species inhabiting cold regions but *Mes. falciformis* Eisen, *Mes. fenestratus* (Eisen), *Mes. primævus* Eisen, and others are found in the arctic regions of the Old World and yet do not possess this peculiarity. Likewise, *Mes. solifugus* Emery and *Mes. niveus* Moore, found in frigid conditions in Alaska, have spermathecæ of the ordinary enchytræid type.

Pigmentation.—Examination of sections of the various regions of the body shows that the color described in foregoing pages has its basis in dark, brownish, non-staining pigment-granules which occur in marked abundance in the body-wall and certain internal organs. The hypodermis bears the principal load, the pigment being distributed through this whole layer. It occurs chiefly in the

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outer ends of the hypodermal cells but may be scattered all through them. Pigment-granules are present in varying amounts in the following internal organs: (1) lymphocytes; (2) chloragog cells; (3) epithelial lining of the ectal, folded end of the spermathecal ampulla; (4) ectal portion of the hypodermal lining of the penial invagination; (5) setigerous glands; (6) lining of the buccal cavity and the pharynx; and (7) ectal ends of the efferent nephridial ducts. Of the above-mentioned internal structures, the lymphocytes and the chloragog cells contain the largest amounts of pigment. Granules do not seem to occur in connection with the nervous systems as is the case in *Mes. solifugus*.

BIOLOGICAL NOTES

The only recorded observation of "snow worms" on Mt. Rainier which the writer has been able to find is given by Moore ('99,p. 142): "In a letter Prof. Russell adds the interesting information that he has observed similar worms on the snows of Mt. Rainier, Wash., thus indicating for them a wide distribution."

All of the information concerning the living form has been supplied by Mr. J. B. Flett, who, as stated before, furnished the writer with the material on which this paper is based. Sexually mature specimens were collected from February 23 to April 5. Whether this represents the seasonal period of sexual maturity is not known. Between the above-mentioned dates, these worms occurred abundantly on the snow-fields of Mt. Rainier, at an elevation of from 2700 to 5600 feet. They also occurred on the snow on the mountain slope in a dense forest of fir and hemlock. These worms have not thus far been found on ice nor on the graciers though they occur on the snow below the ice front and outside of the lateral moraines of Nisqually Glacier. The snow on which they were found is not permanent through the entire season but melts with the coming of summer and it therefore appears that a part of their life history must be spent on or in the ground. During midwinter when the temperature is very low, they are inactive and do not appear on the surface of the snow. Appearance at the surface accompanies the rising temperature in the spring and their activity becomes noticeable when the snow is beginning

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to melt. When placed on hard packed snow during their active period, they are able to bore down through it at will. Under conditions of softening snow, they exhibit a rather efficient locomotion. When taken in the hand, they perform lively squirming movements for a time but soon relax and become quiet. Blue jays and several other species of birds prey upon these worms, picking them off the surface of the snow.

There is the possibility that these enchytræids undergo a color change during the first few days of their appearance. The first specimens, which were collected January 7, are very light yellowish in color and show no evidence, externally or internally, of pigment. Since these specimens are sexually immature, specific identification is not possible but they seem to be the species under discussion. As already stated, the sexually mature form is very dark in color and bears a conspicuous amount of pigment. Mr. Flett is of the opinion that, since the light colored specimens only appear very early in the season and since dark specimens of similar size appear later in the same localities and in approximately the same numbers, this difference in color is probably not a species difference but rather a life history difference in the development of the same species. None of the light colored specimens examined by the writer have been sexually mature but all of the dark colored individuals have either been completely mature or very close to complete sexually maturity. The evidence thus far is circumstantial only and does not justify any definite conclusions. It may be mentioned in this connection that Moore ('99, p. 135), quoting Mr. Bryant, reports what seems to be a similar color change in *Mes. solifugus*.

Nothing definite is known concerning the food of these snow-worms. Mr. Flett reports that the snow over which these enchytræids crawl has a red color, due to a minute, unicellular plant, which, in his opinion, serves as food for the worms. The writer made some examinations of the material contained in the alimentary canal of these worms, and found what seems to be microscopic algæ composed of very minute, globose cells, containing greenish and reddish colors, occurring singly or in clusters, and having the appearance of *Pleurococcus*. This material occurs

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in considerable quantity in the digestive tract and offers evidence leading to the conclusion that the minute snow algæ constitute at least a part of the food of these worms.

A number of the collections made by Mr. Flett contained representatives of the associated animal life. Insects belonging to seven orders, Collembola, Hemiptera, Plecoptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera (several species in five of the orders), one species of Gastropoda, and three species of Araneida were found in the same habitat with the snow-worms. The Collembola (*Isotoma* sp.) occur in enormous numbers, especially on the snow below the glacier, making it black in appearance. In this respect the situation resembles that described by Moore ('99, p. 136) for *Mes. solifugus* which had great quantities of a collembolan, thought to be *Achorutes nivicola,* associated with it. All of these associated animals are black or very dark, except one species of spider and one species of Hemiptera, both of which are largely of a dark red color.

Mesenchytræus solifugus rainierensis n. var.

(Plate XVII, Figs. 20-26)

A collection from the upper snow-fields of Mt. Rainier, made on June 17, 1915, by Mr. Flett, contained seventy-five specimens of any enchytræid, blackish in color but smaller than *Mes. gelidus.* A thorough study of this material, which is in good histological condition, showed that it must be regarded as *Mes. solifugus* Emery. However, several structural characters fail to correspond to the descriptions of Emery ('98a, '98b, '98c, '00a, '00b), Moore ('99), and Eisen ('05). Previously known material of *Mes. solifugus* could not be secured for study but a very careful comparison of the characters of the material from Mt. Rainier with the published descriptions indicates that the deviations are apparently not sufficient to justify the separation into another species. Nevertheless, the variations are so constant that the writer feels convinced that it must be considered as a new variety.

Mes. solifugus was first described by Emery under the name *Melanenchytræus solifugus,* from specimens collected by Filippi on Malaspina Glacier, at the base of Mt. St. Elias, Alaska. The fol-

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lowing year, Moore ('99) published a more extended account of the same species from material collected by Mr. H. C. Bryant, also on Malaspina Glacier. His paper contained not only the results of a careful anatomical study and a quoted account from Mr. Bryant of the habitat and the activities of these annelids, but also an interesting discussion of their relation to the peculiar habitat, with special reference to temperature and light, and an account of the possible function of the dense pigmentation. In the same material, Moore found another form which he described under the name *Mes. niveus*. He also rightly placed *Melanenchytræus* as a synonym of *Mesenchytræus*. Eisen ('05) published a short account of *Mes. solifugus*, specimens of which were collected by Professors T. Kincaid and W. E. Ritter during the Harriman Alaska Expedition on Muir Glacier and on La Perouse Glacier. It thus appears that up to the present account *Mes. solifugus* has been known only from glaciers in a very limited region of Alaska.

Definition.—Length, 12-18 mm., average about 15 mm. Diameter, about 0.52 mm. Somites, 51-60, average 55. Color, very dark brown to black. Prostomium rounded, smooth, blunt. Head pore at tip of prostomium. Setæ sigmoid, abruptly bent at distal ends; uniform in size and shape; in anterior fourth of body, 2 setæ, rarely 3, per bundle in lateral rows; in remainder of body, 1 seta, rarely 2, in lateral rows; in anterior third of body, 2-4 setæ, usually 3, per bundle in ventral rows; 2 setæ per bundle in ventral rows in remainder of body. Clitellum absent. Brain but very slightly longer than wide; anterior margin deeply emarginate, posterior margin shallowly concave, lateral margins approximately parallel. Origin of dorsal blood-vessel in XIII-XIV; small cardiac body present. Nephridia with small, slender, anteseptal part and large, irregular, lobate postseptal part; origin of efferent duct on ventral surface of latter very near posterior end. Spermiducal funnel moderate, cylindrical, tapering slightly towards origin of sperm duct; length about three times the maximum diameter; collar absent. Sperm duct very long, extending caudad within ovisac to XX; masses of coils in XIII-XIV; diameter uniform throughout. Ovisac extending from XII/XIII to XX, bifurcating in XIII or XIV. One pair of sperm sacs extending from XI/XII to about

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XV; within ovisac. Penial bulb large, subglobular; well-developed, fusiform atrium with five atrial glands; numerous groups of multicellular glands within bulb. Spermathecæ confined to V; duct short, cylindrical, numerous unicellular glands externally; ampulla but little greater in diameter than duct, elongate, tapering slightly entad and uniting in posterior part of V with lateral aspect of digestive tract; two opposite, elongated, cylindrical diverticula reflected caudad.

The description is based on twenty-seven sexually mature specimens. Forty-eight other specimens of uncertain sexual maturity were examined in the study of external characters. The type and most of the paratypes are in the collection of the writer. Paratypes have been deposited in the collection of the United States National Museum and in the collection of Professor Frank Smith.

The habitat of this species will be described in another part of the paper.

External Characters

The body is elongate, cylindrical, smooth, and uniform in diameter except at the extreme anterior and posterior ends where there is a slight but gradual tapering. The length of the alcoholic specimens varies from 12 to 18 mm., the average length of twenty-seven sexually mature specimens being approximately 15 mm. The diameter varies from 0.47 to 0.63 mm., the average being about 0.52 mm. Except in the extreme anterior region, the external segmentation is indistinct, the surface being smooth and the intersegmental grooves difficult to detect. As in *Mes. gelidus,* external examination shows the presence of ruptures in some of the intersegmental grooves, thus producing whitish rings between somites. Longitudinal sections show that these ruptures are breaks in the cuticula and hypodermis and the underlying muscle-layers are thus exposed, producing the whitish band. Such ruptures are present in almost every specimen sent to the writer and occur uniformly in the middle region of the body, the anterior and posterior ends only being invariably free from them. No information is available at present concerning the origin of these interruptions. Moore ('99, p. 126) reports a similar feature in some of the specimens

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of *Mes. solifugus* which he examined. He also states that Mr. Bryant, the collector who secured the material on Malaspina Glacier, Mt. St. Elias, and furnished data on the living animal, informed him that these white or yellow bands "were present when the worms were collected". On a later page (p. 135), Moore quotes directly from an account by Mr. Bryant, a part of which is as follows: "Some of the specimens I obtained had also distinct whitish bands around their bodies." From this account it might appear that these light bands occur in the living worms. Whether it is true of *Mes. gelidus* and *Mes. solifugus* var. *rainierensis* remains to be discovered. It seems unlikely, from an examination of the available material, that these interruptions are normal. Longitudinal sections show ragged edges of tissue about these interruptions, a fact which seems to constitute evidence against such a view. The appearance suggests the killing and preserving operations as the cause, although none of the specimens showed signs of extreme contortion or severe treatment.

The external surface of each somite is smooth and free from ridges, secondary constrictions, or striations of any sort. The number of somites varies from 51 to 60, the average being about 55. Except at the extreme anterior and posterior ends, the somites are of approximately uniform width. The color of the entire body is very deep brown to black, except for the above-mentioned whitish bands between somites. To the unaided eye, the specimens are black but under magnification, using transmitted or reflected light, they are a deep, rich brown. The distribution of the color is almost uniform throughout the body. Specimens, cleared and mounted *in toto,* show on the surface, except on XI–XIII, innumerable, minute, polygonal areas, usually hexagonal, each of which has a long and short dimension, at right angles to each other, the former extending in the direction of the circumference of the body. Each area contains in its center a light, oval spot.

Certain special, external areas are distinctly lighter in color than the surrounding surface. The tip of the prostomium and the lateral parts of the groove 0/I are yellow and offer contrast with the adjacent parts. The ectal opening of the spermatheca in IV/V is surrounded by an ovate, yellowish area which, however, is not as

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distinct as in *Mes. gelidus*. The posterior end of the last somite and the immediate vicinity of the penial bulb invaginations are also much lighter than surrounding parts.

These specimens lack the conspicuous, elliptical, swollen areas about the ectal openings of the spermathecæ which Moore ('99, p. 125; Figs. 1, 2) found in the Malaspina Glacier specimens. Such areas are represented only by the lighter color in the immediate vicinity of the openings described above. Emery and Eisen make no mention of these areas.

A distinctly differentiated clitellum seems to be absent. The region of XI–XIII differs from the adjacent somites only in the obscurity of the intersegmental grooves and the slightly increased body diameter in that part containing the penial bulbs. Transverse and longitudinal sections of sexually mature specimens show no increase in the thickness of the hypodermis. The apparent absence of a clitellum causes some difficulty in distinguishing sexually mature individuals in superficial examination but in the material on which this paper is based it has been found that all specimens showing distinctly protruding penial bulbs are sexually mature. The uniform absence of a differentiated clitellum might cast some doubt, at first thought, upon the sexual maturity of all of the specimens but serial sections and dissections have demonstrated the presence of spermatozoa in the spermathecæ, developing ova and spermatozoa in the storage sacs, and well developed ovaries and testes, thus furnishing proof of sexual maturity. Moore ('99, p. 125) reported that "In none of the specimens examined (about twenty in number) is the clitellum very distinctly developed, but on the contrary is thin and scarcely extends beyond the limits of the twelfth somite." Eisen ('05, p. 59) found it "probably confined to XII."

The head pore is distinct and located very near the tip of the prostomium. The external opening is slit-like in appearance, transverse in position, and surrounded by a very narrow, yellowish area.

The setæ are distinctly sigmoid and arranged in fan-shaped bundles, two lateral and two ventral. Those of a bundle are of approximately equal development. Anterior to the penial bulb invagination, the lateral bundles contain 2 setæ, very rarely 3;

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posterior to that region, they contain 1 seta, very rarely 2. Anterior to the vicinity of XX, the ventral bundles contain 2-4 setæ, usually 3; posterior to that region, they contain 2 setæ. The setæ are somewhat distinctive in the very abrupt bend at the distal, exposed end (Pl. XVII, Fig. 24). The proximal end is broadly curved and deeply imbedded in the body-wall. A comparison with the descriptions of Moore and Eisen shows that the number of setæ per bundle which they report is greater than has been found in the Mt. Rainier material. In the latter, the writer has found but a single bundle which contained five setæ. It is not possible to determine whether the abrupt bend at the distal end of each seta described above is a characteristic only of *rainierensis* since Moore ('99, p. 126) states that in his Alaskan specimens "The setæ have the form usual in the genus, being feebly sigmoid and arranged in fan-shaped bundles, but are mostly imperfect, owing to the points being worn or broken off." He also reports that "Enlarged setæ are found in the ventral bundles of XI; these are about one-third longer and much thicker than the others." Such enlarged setæ have not been found in the specimens from Mt. Rainier. Emery ('00b, p. 225, Fig. 10) states that "The *chaetae* are slightly sigmoid, more markedly bent at their apical end (Fig. 10). They are about a third longer in the posterior half of the body than in the anterior segments, as it appears by comparing Figs. 12 and 13. Each bundle consists of four nearly equal chaetae. The ventral bundle is absent in the 12th (clitellar) segment, which receives the opening of the sperm-duct." From this description it might appear that the setæ resemble closely those of *rainierensis* but the figure which accompanies Emery's description portrays setæ quite different from those found in the Mt. Rainier specimens. Instead of being very slender, approximately uniform in diameter, distinctly sigmoid, broadly curved at the proximal end, and abruptly curved at the distal end, they are short and very stout, having, in the posterior bundles, a diameter of one-sixth the length. The diameter increases from the distal end to the opposite extremity which is almost straight. The distal end is very acute.

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Internal Characters

In many respects, the internal anatomy corresponds to that described, by previous observers, in the Alaskan form and for that reason only new data and the features which present differences will be discussed.

Brain.—Dissections and frontal sections show the brain (Pl. XVII, Fig. 22) to correspond, in a general way, to the accounts of the same organ in Alaskan specimens. It resembles more closely the figure and description given by Moore, differing in being a little longer than broad and in having the posterior margin distinctly but shallowly concave. However, these are minor differences. It also resembles the figure given by Emery ('00b, Fig. 2) except that the concavity of the anterior margin is much deeper. A typical measurement is as follows: maximum length, 0.137 mm.; maximum width, 0.129 mm.; maximum thickness, 0.043 mm. A supporting strand extends latero-caudad from each latero-caudal angle to the body-wall.

Dorsal Blood-vessel.—According to the previous descriptions of *solifugus,* the origin of the dorsal blood-vessel occurs in XII. In the Mt. Rainier specimens, this vessel becomes distinct from the perivisceral blood-sinus in the posterior part of XIII or the anterior part of XIV. In none of the specimens examined did it arise in XII. An inconspicuous cardiac body is present.

Septal Glands.—In position, the septal glands agree with the description by Moore ('99, p. 127) but cannot strictly be called "large". Eisen ('05, p. 59) found the "Septal glands small" but the position is not indicated. Of the two indefinite terms, the latter is more nearly correct for the specimens studied by the writer.

Emery ('98a, pp. 110-111) includes the following statement in his original description of *solifugus*: "Nei segmenti 4-8 la cavita viscerale è in gran parte occupata da ghiandole unicellulari, i cui lunghi e sottili condotti sboccano all' esterno in vicinanza dei gruppi ventrali di setole." Michaelsen's description of *Mes. solifugus* ('00, p. 87) contains the following: "Liebeshöhle des 4.-8. Segm. von grossen einzelligen Drüsen erfüllt (Kopulationsdrüsen?), die in der Nähe der ventralen Borstenbündel ausmünden." This statement, evidently taken from Emery, contains the tentative suggestion

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by Michaelsen as to the character of the glands. Emery ('00b, pp. 226-227) in his later and more complete paper, describes these glands as follows: "In the segments 4-8, the most part of the body-cavity is filled by *unicellular glands* (Fig. 11 gl); their very thin excretory prolongations form numerous threads directed towards the ventral side, which can be easily followed on the sections to the sides of the ganglion chain. Their thinness and flexuous course make it difficult to follow them to their end on the surface of the skin. I believe that they converge towards the bundles of chaetae of the ventral series. As Mr. Michaëlsen writes me, these glands may be regarded as morphological equivalents to those gland-cells which in other Enchytraeids are related to the chaetae of the genital segments. In Melanenchytraeus, I don't think that these glands have any relation to the function of reproduction, because I find them no less developed in immature specimens." No mention of such special unicellular glands is found in the descriptions of Moore and Eisen and the writer has found no evidence of them in his material. Since the position of these unknown glands coincides with the position of the septal glands, as described by Moore and as found by the writer in Mt. Rainier specimens, it might be suspected that the two have been confused. Emery ('00b, Fig. 11) figures these glands as they appear in a longitudinal section of the body and although the component cells are not associated in close, compact masses, they are aggregated into rather loosely associated groups and columns with their prolongations approaching each other and extending in the same directions. The appearance of the whole very strongly suggests loosely constructed septal glands and since Emery worked with a very few sexually mature specimens which were not in the best state of preservation, the writer is inclined to suspect these masses of "unicellular glands" of being the usual series of septal glands.

Nephridia.—The nephridia (Pl. XVII, Fig. 25) agree closely with previous descriptions. A well-developed, ciliated nephrostome, borne on a slender base, comprises the anteseptal part. The postseptal region is enlarged, somewhat lobate, and slightly compressed. The structure of the interior is of the usual mesenchytræid type. The efferent duct arises from the ventral surface of the postseptal

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part near the caudal end. In the specimens examined, there is considerable variation in the size and shape of the nephridia in different regions of the body but the general structure is fairly constant. The first pair is located on VI/VII. Moore ('99, p. 127) found nephridia "in every somite posterior to VII." No nephridia have been found on XI/XII and XII/XIII in the Mt. Rainier specimens.

Lymphocytes.—Lymphocytes are very scanty, in some specimens almost absent. A few scattering cells occur in the anterior region and occasional ones more caudad. They are small, elliptical, nucleated, and contain pigment-granules.

Spermiducal Funnel.—The spermiducal funnels (Pl. XVII, Fig. 23) depart somewhat from Moore's descriptions of Alaskan specimens of *solifugus*. Instead of being constricted at the middle and bent upon themselves so that the free end is directed caudad, they show only a slight diminution in diameter and a slight bend at the middle so that the free end is directed cephalo-dorsad. A much greater disagreement is found when comparison is made with Emery's figure ('00b, Fig. 16) of the funnel which is represented as a very short, funnel-shaped organ which is broader than long. However, Emery states that his figure was drawn from a reconstruction. The length is about three times the maximum diameter, a typical set of measurements being as follows: length, 0.344 mm.; diameter, 0.113 mm. The collar is entirely absent.

Sperm Duct.—The sperm duct is very long and of approximately uniform diameter. Because of its coiled and contorted condition, the exact determination of its length has not been possible. It extends from the end of the spermiducal funnel into the ovisac; forms contorted masses in XIII and XIV; continues caudad within the ovisac to XX; thence is reflected cephalad to XII to unite with the atrium. Throughout the entire course it is characterized by numerous coils and curves. Its extent is represented by the combined length of sixteen somites plus an allowance of the length of several somites to compensate for the contortions. Except for a very small part at the end of the spermiducal funnel and at the union with the penial bulb, the entire duct is contained in the ovisac, and does not, according to the writer's observations, enter either of the sperm sacs. Moore ('99, p. 129) found that, in his material,

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the sperm duct on the right side lies within the ovisac and extends to the region of XVIII, but the one on the left side lies free in the body-cavity, extending also to the region of XVIII. Eisen's description contains no account of these ducts.

Sperm Sacs.—The sperm sacs are a pair of caudal evaginations of XI/XII, one on each side of the median line, which lie latero-ventrad of the digestive tract and extend to XV or XVI. In the specimens examined, they are rather small, but both are of about equal development. Both lie within the ovisac and both contain masses of developing spermatozoa. The structure of the walls of these sacs is identical with that of the septa.

Moore ('99, p. 128) found a different condition in Alaskan material. The sac on the right side is almost rudimentary, extending only into XII, while the one on the left side is well developed, extends caudad to XX, and is apparently not contained within the ovisac. Other descriptions do not include data on the structure and relations of these sacs. Allowing for a liberal variability, such differences seem rather large for intra-species variation.

Ovisac.—The ovisac is formed by the caudal outgrowth of XII/XIII. It arises as a single, well-developed sac and extends caudad, ventrad to the digestive tract, to the posterior part of XIII or to XIV where it bifurcates, forming two branches, one on either side of the median line, which extend to the vicinity of XX. There is some variation in the length since specimens were examined in which the caudal terminations were in XXII, while in others they were in XIX. As stated above, the ovisac contains the sperm ducts and the sperm sacs. In addition, it contains masses of developing ova. Here, again, is an interesting divergence from the condition described by Moore ('99, pp. 129-130) who found, in the Alaskan material, that the ovisac is single throughout its entire length and contains only the right sperm duct and the masses of developing ova. The length, however, is very similar in both cases.

Penial Bulb.—In the general plan of structure, the penial bulb (Pl. XVII, Figs. 20, 26) agrees with the description by Eisen ('05, p. 60) for Alaskan specimens. However, a number of differences exist in some of the finer structural detail. The whole

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organ is of the mesenchytræid type as described by Eisen ('05, p. 7) and consists of three sets of structures, viz., the penial bulb proper, the atrium and its associated parts, and the accessory glands. The whole organ is conspicuous in size so that both bulbs occupy the greater part of the cœlom in that region.

The penial bulb proper is situated on a deep invagination which, in transverse section of the body, is slit-like but in longitudinal section appears as a narrow channel leading, at its inner extremity, into an expansion which takes the form of a chamber, narrow in dorso-ventral dimension but much wider in transverse dimension. This inner chamber caps the ental part of the invagination like the top to a mushroom. Structurally, the wall of the invagination is essentially a continuation of the body-wall. The ectal end of the sperm duct connects with the inner, expanded chamber. The body of the bulb is composed of (1) a large number (over twenty-five) of multicellular glands, (2) scattering unicellular glands, (3) a large number of muscle-strands, and (4) the ectal end of the sperm duct. The multicellular glands are pear-shaped, the expanded ends being entad of the invagination. Each is composed of a number of gland cells aggregated in the expanded ends and a narrow, tapering extension, composed of the elongated ends of the gland cells, which connects with the penial invagination. None of these glands opens into the sperm duct. The unicellular glands are sessile, very inconspicuous, and are scattered singly about the invagination into which they apparently open. They are the only glands which occur laterad of the invagination. In the interior of the bulb, muscle-fibers extend in different directions. They lie between the various glands and many of them are attached to the wall of the invagination. They vary in size from very fine threads to rather strong strands. The ectal end of the sperm duct, which is contained within the body of the bulb, has the usual structure except that it is surrounded by a very strong, longitudinal muscle-coat which is apparently derived from the longitudinal muscle-layer of the body-wall.

Within the penial bulb but near its ental surface, the sperm duct begins to expand into the atrium so that a small portion of the ectal part of the latter is included within the envelope of the

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bulb. The atrium is a stout, fusiform organ, about 0.3 mm. long and 0.13 mm. in maximum diameter. Its histological structure is very similar to that indicated in Eisen's figure ('05, Pl. VIII, Fig. 1), except that the epithelium on the surface does not seem to be as distinct and thick. At the ental end of the atrium is a set of about five large, club-shaped, multicellular atrial glands, arranged in a whorl. These glands extend into the cœlom and are conspicuous organs in sections of that part of the body.

A number of large, multicellular, pear-shaped, accessory glands are present. They lie just outside of the envelope of the bulb and open into the invagination, commonly at its origin. In general structure, they resemble the multicellular glands within the penial bulb. The surrounding envelope is very delicate and it is difficult in some of the specimens to determine just how many of the glands at the edge of the bulb are to be classed as accessory.

The structure of the penial bulb and its associated parts in *solifugus* has been briefly described by Moore ('99, p. 129) and Eisen ('05, pp. 59, 61) as it occurred in the Alaskan material which they examined, and a comparison with the Mt. Rainier specimens is of interest. Moore states that "Before entering the atrium in somite XII the recurrent limbs of the sperm ducts expand into narrow fusiform sacs (.....), having glandular, epithelial and muscular walls, which receive the ductules of a group of unicellular spermiducal glands. This structure probably serves to form and eject the spermatophores. A narrow curved duct, which is also provided with some unicellular glands, perforates the mesial wall of the atrium and opens into its lumen. Unlike the remainder of the male efferent apparatus, the atrium (.....), is, in part, of ectodermal origin, as is indicated by the pigmented lining epithelium. It is a spheroidal thick-walled partly eversible sac, with an internal cavity having a mushroomlike shape in the retracted organ. Its walls are composed of a cuticle-covered, rather deep, pigmented and perhaps glandular epithelium, surrounded by a thick muscular layer in which the fibres are partly longitudinal, but largely radial, especially about the place of entrance of the sperm duct. A number of groups of unicellular glands are attached to the organ, and probably empty into its lumen."

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At first sight, it might appear that a radical difference exists between the structure of the Alaskan and Mt. Rainier specimens. In the first place, a difference in the use of terminology is evident. Moore applies the term *atrium* to the structure which the writer and others call the *penial bulb,* and speaks of the expansion of the sperm duct just entad of the bulb as "narrow fusiform sacs". The writer follows Michaelsen ('00, p. 9) and Eisen ('05, p. 4) in using the term *atrium* to designate the enlargement of the sperm duct which is situated just entad of its union with the penial bulb. Apparently, Moore regards the atrial glands and the multicellular glands within the penial bulb as "groups of unicellular glands" rather than multicellular glands as described by the writer. If this interpretation of Moore's description is in error, then the structure of the penial apparatus in the Alaskan specimens is very different from that described in the present paper. No distinction is made in the above-quoted description between the penial glands within the bulb and the accessory glands.

Eisen ('05, pp. 59, 61) describes the penial apparatus in *solifugus* as follows: "A large atrium in which opens about eight atrial glands of large size. Many large accessory glands open along the base outside of the penial bulb. About fifteen penial glands inside the penial bulb. . . . . The accessory glands, which are characteristic, open along the base of the penis outside of the bulb. They are long and of trefoil shape, with enormous long narrow ducts." It will be noted that Eisen found a greater number of atrial glands in his material. Furthermore, the accessory glands are evidently much longer and the number of penial glands in the bulb greater than in *rainierensis*.

Emery ('00b, pp. 227-228, Fig. 16) describes the penial apparatus as follows: "The last tract [Sperm duct] forms a spherical bulb (*a*), but before reaching it the tube presents a fusiform swelling (*c*), whose wall is very thick and made of long cells, directed radially on the transverse section, the lumen being not widened. Bundles of prostatic (spermiducal) glands (*b*) are related to the bulb; another little group of glands (*e*) lies around the tube, above its fusiform thickening." Some difficulty is experienced in interpreting this description, expecially when the figure is con-

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sulted. The spherical body of the bulb and the atrium agree with the other descriptions but the "prostatic glands" are represented as large, rather numerous, lobular organs which lie out in the body cavity, opening into the penial bulb much as do the corresponding organs in *Mes. gelidus*. The other group of glands referred to is of uncertain identity, judging from either the description or the figure. Emery's figure was made from the reconstruction of a series of sections and is possibly not fully dependable, although it seems improbable that a mistake could have been made in the matter of so large a group of glands as those which he calls prostatic. Assuming that his observations have been fairly correct, the penial apparatus differs from those described by Eisen and Moore as well as from *rainierensis*.

Spermathecæ.—The spermatheca, in all of the specimens examined, consists of (1) a short, stout, cylindrical duct, covered externally, in part, by numerous attenuated, unicellular glands, (2) two almost oppositely placed, elongated, cylindrical diverticula which are directed caudad, and (3) an elongated, slightly curved ampulla which decreases slightly in diameter towards the ental end and joins the digestive tract independently on its lateral aspect. The character of the area surrounding the external opening of the duct has already been discussed.

Certain variations are apparent when these organs are compared with the descriptions of *solifugus* from other localities. Moore ('99, pp. 130-131) found three spermathecal diverticula. He also found that the ampullæ of the two spermathecæ unite to form a short duct before joining the digestive tract on its dorsal side. No mention is made of unicellular glands on the duct. Eisen's description ('05, p. 60; Fig. 32b) agrees with that of Moore in almost every respect. However, his figure shows, on one of the spermathecæ, one diminutive and three equally developed diverticula, indicating a possible variation, although no mention is made of it in his description. Emery's original description of *solifugus* ('98a, pp. 110-111) contains the following statement: "I ricettacoli del seme non communicano con l'intestino; sono in continuità l'uno coll', altro ed hanno ciascuno, alla base della loro ampolla, due o tre diverticoli." His more complete paper ('00b) corroborates this

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statement. It appears, from the above, that specimens with as few as two spermathecal diverticula were found. The reported absence of the communication of the ampulla with the digestive tract may be an error as has been suggested by Moore. Emery ('00b, p. 230) re-examined his material and found no connection but states that the spermathecæ lie in close contact with the intestine and points out the possibility that his single sectioned specimen might be not fully mature or abnormal.

Pigmentation.—All of the specimens from Mt. Rainier are deeply pigmented. Microscopic examination of sections of all parts of the body shows that the hypodermis bears a heavy load of minute, dark brown, non-staining pigment-granules, especially in the outer ends of the cells. This pigmentation extends to the internal organs. The chloragog cells, which first appear in IV and are present throughout the remainder of the body, are heavily loaded with pigment. It also occurs in the glands surrounding the bases of the setæ, in the lymphocytes, in the lining of the buccal cavity and the pharynx, in the spermathecal duct, in the lining of the penial invagination, in the nerve cord, and in the brain. It will be noted that this distribution of pigment corresponds closely with that described by Moore ('99, p. 127) for specimens of *solifugus* from Alaska. Eisen ('05, pp. 59, 60) states that the pigment is distributed in the body-wall and also in most of the internal organs "even in the ganglia and the brain" but does not specify further detail.

Summary of Comparisons.—The variety, *rainierensis,* differs from the Alaskan specimens of *solifugus* in the following respects: (1) the smaller number of setæ per bundle, (2) the absence of enlarged setæ on XI, (3) the origin of the dorsal blood-vessel in XIII-XIV, (4) the concavity of the posterior margin of the brain, (5) the constant presence of only two diverticula on the spermatheca and the independent opening of each spermatheca into the lateral wall of the digestive tract, (6) the shorter accessory glands, the smaller number of atrial glands, the larger number of multicellular glands within the penial bulb, and the straighter spermiducal funnel, and (7) the complete enclosure of the sperm sacs and sperm ducts by the ovisac. All of these differences seem

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to be constant and are sufficient to raise the question as to whether they are too wide to be considered within the range of intra-species variation. However, they are differences of narrow margin and when compared with the sum total of the points of agreement with *solifugus* as described, the writer is convinced that the Mt. Rainier material is not a distinct species but can be considered only as a new variety.

BIOLOGICAL NOTES

As stated before, all of the information concerning the living specimens has been furnished by Mr. Flett. He found *Mes. solifugus* var. *rainierensis* abundant on the higher snow-fields and glaciers of Mt. Rainier in early summer. The collection which has furnished the material for the preceding description was made on June 17, 1915, at an altitude of 7,500 ft. Nothing is known concerning the winter or early spring conditions since at those seasons collections at such heights are not possible. These worms occur on snow-fields which seldom thaw during the summer and they evidently pass the entire existence, generation after generation, in the snow and ice. Mr. Flett states that on one occasion he has seen what he thought was this worm at an elevation of only 6,000 feet where the snow melts and grass and flowers grow in profusion during three or four months of the year. However, this was an unusually low altitude for these worms since they occur regularly and more abundantly much higher up the mountain on the permanent snow-fields and on the snow and ice of the glaciers. The writer has not had the opportunity of studying specimens from an altitude as low as 6,000 feet, but if the worms seen at that level are *solifugus* var. *rainierensis*, as Mr. Flett thinks they are, it appears that those individuals which chance to be developed at the unusually low levels must pass a part of the life history in midsummer on or in the ground.

On the glaciers, these worms coil up so as to appear as small spherical black dots on the snow or solid ice and it requires a considerable exposure to sunshine to warm them up to the active stage. According to the observations of Bryant (Moore, '99, p. 134), the specimens of *solifugus* on Malaspina Glacier, Mt. St.

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Elias, "remain on the surface during the night; but when the sun appears in the morning they again burrow into the snow". This does not agree with the observations of Mr. Flett for the variety *rainierensis* since he has noticed no tendency on the part of the worms to avoid sunlight, the warmer the day the more active they become, this activity being manifested on the surface. The period of greatest activity is usually from the middle of the afternoon to about five or six o'clock.

No data on the associated life are available except the statement by Mr. Flett that snow algæ ("Sphærella nivalis") occur in great abundance and that spiders and snow-fleas (Collembola) are present.

The question of the food of a worm living in such a habitat is of considerable interest. Obviously, in the case of those individuals living on the permanent snow-fields and the ice of the glaciers, the number of substances serving as food are extremely limited. Emery ('00b, p. 226) found the contents of the intestine of Alaskan specimens, especially in the posterior part, to consist of "very fine crystalline mineral detritus, which seems to be the ordinary food of this worm". An examination of the intestinal contents in the Mt. Rainier specimens yielded practically no definite data. A certain amount of what seems to be fine, angular, mineral particles is present. However, the conspicuous content was a more bulky material, which the writer was unable to identify. In some respects, it has the appearance of partly disintegrated vegetable matter. This material was found in every specimen examined and evidently represents a part of the usual food.

GENERAL CONSIDERATIONS ON SNOW-FIELD AND GLACIER WORMS

Temperature Relations.—A striking thing about the environment of these enchytræids is the very low temperature of the medium in which they live. They exist and carry on their life processes under freezing temperatures and in a medium of snow and ice. Species, such as *Mes. gelidus,* which regularly occur at an altitude below the limit of permanent snow-fields evidently pass

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a part of the life history on or in the ground, but aside from the midsummer months they are covered with snow and live in the latter or else in the earth beneath it. In either case, they are passing the greater part of the year in cold conditions, appearing in the melting snow in early spring. On the other hand, *solifugus*, which regularly inhabits the permanent snow-fields and the ice of the glaciers, spends its entire existence, generation after generation, under these conditions. Apparently, it must deposit its eggs in the snow, on the ice, or in the small pools of ice water which sometimes occur on the surface of the lower parts of the glaciers, and the young worms must be able to withstand the rigid conditions and successfully solve their problems of maintenance.

Pigmentation.—Nothing is known concerning the rôle of the large amount of pigment. Reference has already been made to the speculative discussion of this problem by Moore ('99, pp. 135-142). It may be related to the maintenance problems of heat or light, or both, but until some careful observations and experiments are made no definite conclusions can be drawn. If such pigmentation were confined exclusively to glacier forms the circumstantial evidence might be stronger in favor of a theory that it is an adaptation to some of the factors peculiar to that environment, perhaps light and temperature, but the fact that species other than glacier forms are known (*Mes. harrimani* Eisen, *Mes. obscurus* Eisen, *Mes. maculatus* Eisen, and others) which possess similar pigmentation throws some doubt on such an assumption. If it be true, as is suspected in some cases, that the younger stages of these glacier worms are not pigmented, a critical study of the life history may be necessary to the accurate solution of this problem.

Other Snow-field and Glacier Worms.—It is possible that future investigations of the northern glaciers and the arctic snow-fields will reveal still other enchytræids which occupy these frigid habitats and extend the knowledge of the distribution of the species already known. Certain arctic explorers make mention of the presence of "worms" on the snow and ice and while no hint is given of their identity, it is quite possible that they are enchytræids. Mr. Flett states that he found a snow-worm in the Olympic Mountains which occurred in enormous numbers, making the snow black,

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and which resembles *Mes. solifugus* var. *rainierensis* in size and general appearance, but since material has not been secured its identity is unknown. Among the collections from Mt Rainier, there is at least one other enchytræid which appears to be distinct from those described in this paper but has not been carefully studied.

Thus far, all of the known glacier worms belong to the genus *Mesenchytræus*. While our knowledge concerning the geographical distribution of *Enchytræidæ* is incomplete, it appears fairly certain that the family is one of temperate and frigid distribution. Ude ('01, p. 23) found, among other things, that available data showed that "Die Gattungen *Mesenchytraeus* und vielleicht *Henlea* (vergl. *H. ventriculosa* (Udek.) lassen in ihrer Verbreitung Cirkumpolarität vermuten." Certain other genera (*Bryodrilus, Lumbricillus,* and others) have representatives in the arctic zone but none of them seem to have been reported as living continuously in snow and ice. Whether this habit is confined to *Mesenchytræus* is a problem for future investigation.

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EXPLANATION OF PLATES

Abbreviations

ac. gl.,	accessory gland.
atr.,	atrium.
atr. gl.,	atrial glands.
cut.,	cuticula.
ec. op.,	ectal opening.
hyp.,	hypodermis.
in. pen. gl.,	intra-penial glands.
lum. amp.,	lumen of ampulla.
lum. div.,	lumen of diverticulum.
ov's.,	ovisac.
pen. b. i.,	penial bulb invagination.
pen. ch.,	penial chamber.
pen. gl.,	penial glands.
pen. po.,	penial pore.
r. m.,	retractor muscle.
sp. d.,	sperm duct.
sp'r.,	spermatheca.
sp'r. d.,	spermathecal duct.
sp. s.,	sperm sac.
I-XXXV.,	somites.

Plate XIV

Mesenchytræus gelidus

Fig. 1. Diagram of XI–XXXV, showing position and extent of ovisac, sperm sacs, spermiducal funnels, and sperm ducts. Broken lines indicate omission of somites.

Fig. 2. Diagram of I–X, showing extent of the exceptionally large spermathecæ. The broken lines across spermathecæ in posterior part of VIII indicate most anterior observed termination of these organs.

Fig. 3. Longitudinal section through ectal region of spermatheca.

Fig. 4. Lymphocytes, indicating abundance of pigment-granules in cytoplasm.

Fig. 5. Chloragog cells. Pigment-granules distributed through cells.

Fig 6. Longitudinal section through ectal opening of spermatheca, showing unicellular glands which occur in connection with end of spermathecal duct.

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PLATE XV

Mesenchytræus gelidus—cont.

Fig. 7. Spermatheca.
Fig. 8. Spermatheca.
Fig. 9. Setæ. Bundle from ventral row.
Fig. 10. Brain.
Fig. 11. Penial bulb and associated structures.
Figs. 12–13. Spermathecæ from one specimen, drawn to same scale, showing a rather exceptional form of variation in development.
Fig. 14. Nephridium.
Figs. 15–17. Diagrams of different forms of spermiducal funnel.
Fig. 18. Nephridium.

PLATE XVI

Mesenchytræus gelidus—cont.

Fig. 19. Longitudinal section through penial bulb.

PLATE XVII

Mesenchytræus solifugus var. *rainierensis*

Fig. 20. Penial bulb as it appears in transverse section of body.
Fig. 21. Spermatheca.
Fig. 22. Brain.
Fig. 23. Spermiducal funnel.
Fig. 24. Setæ.
Fig. 25. Nephridium.
Fig. 26. Penial bulb and associated structures.

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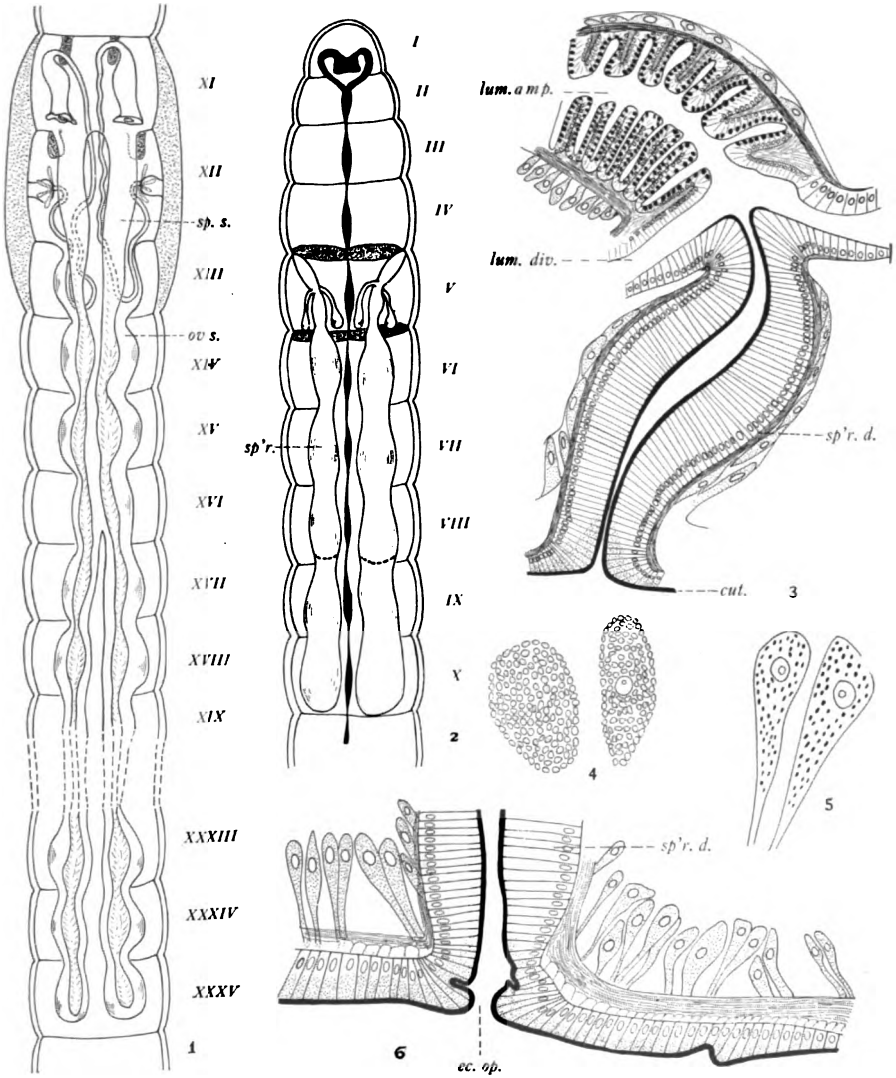

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PLATE XIV

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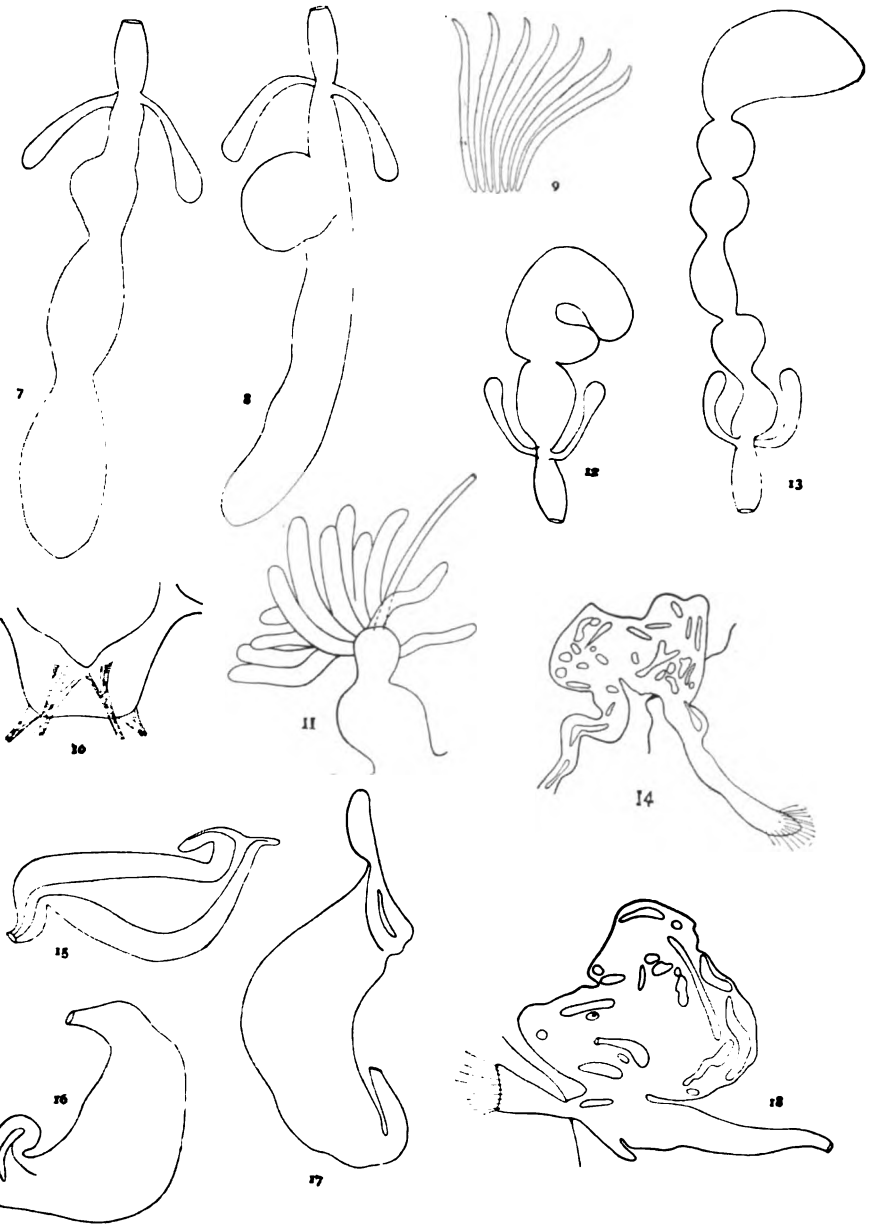

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PLATE XV

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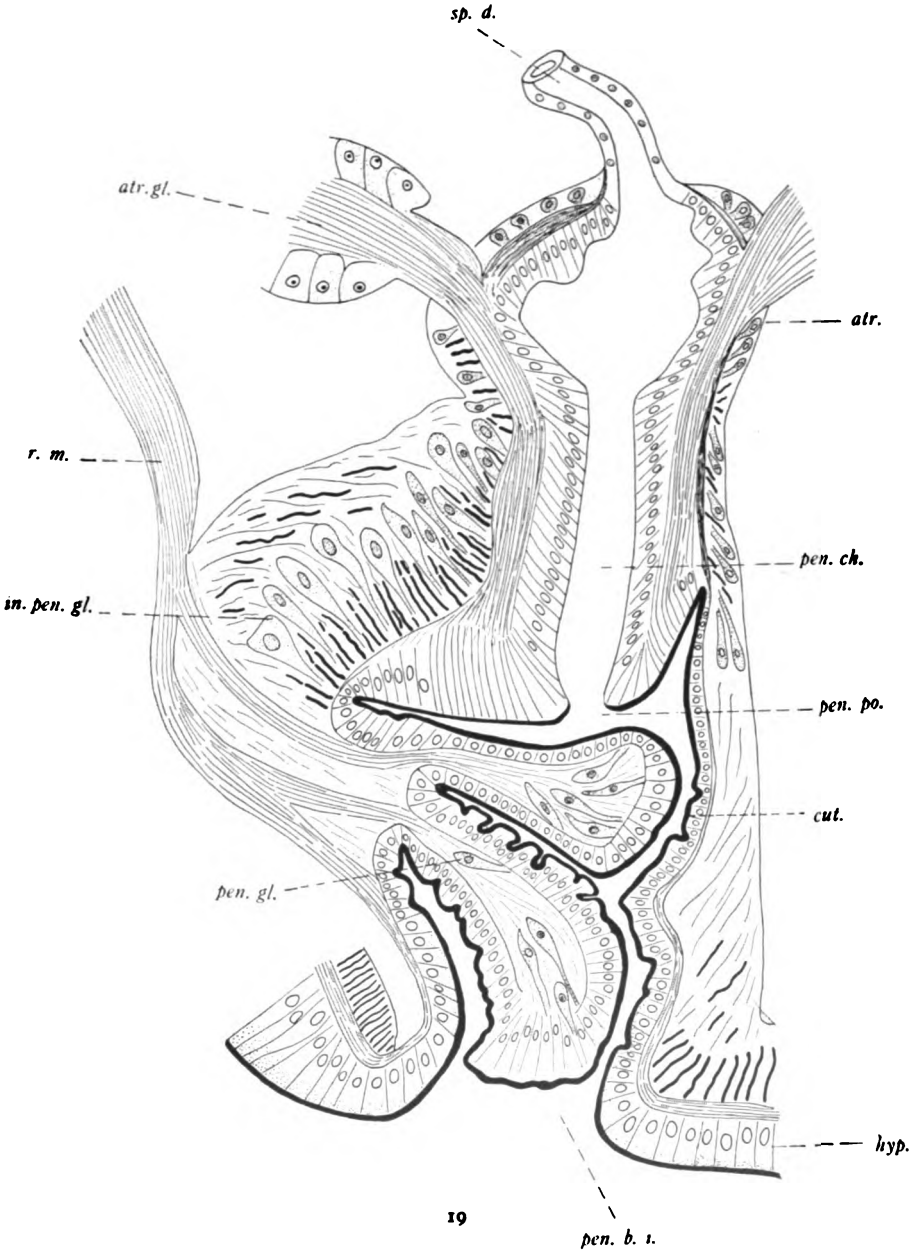

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PLATE XVI

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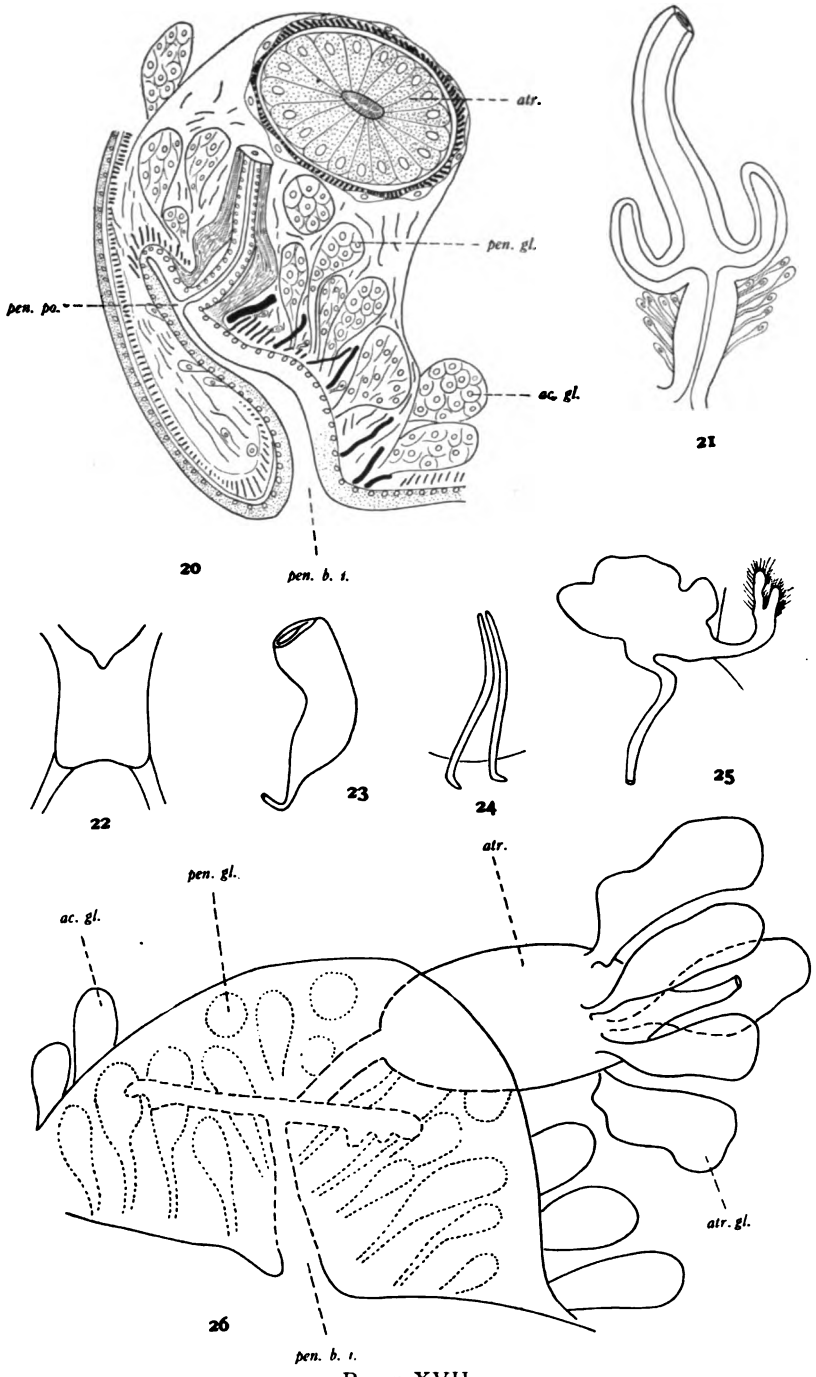

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PLATE XVII

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ON THE SO-CALLED INTESTINAL GLANDS IN NECTURUS MACULATUS

By HAROLD TUPPER MEAD

From the Zoological Laboratory of the University of Chicago

Introduction.

Imbedded in the submucosa and connected to the mucosa in the intestines of Necturus are groups of cells whose function has not been determined. The finer structure and function of these groups of cells constitute the object of the present work.

Groups of cells of similar character have been described in Proteus, the European Salamander, Newt, Triton and Amblystoma, but have never been mentioned in connection with other animals. It is of interest to note that forms as closely allied as Rana do not possess these groups of cells, nor, according to a recent paper by A. M. Reese, does the Alligator. Since the forms mentioned above represent two of the three suborders of Urodeles it is probable that they are characteristic of Urodeles only.

The function of these groups of cells may be similar in the different animals which possess them, but two views have been held as to their function. One view is that they are glands discharging into the intestine. This other view is that they serve as proliferation centers where the epithelial cells which constitute the musoca are produced.

Various names, more or less suggestive of function, have been applied to these structures, such for example as "gland," "Sprossen" (sprout), "Bud," "Zapfen" (spigot) and "Ersatzzellen" (compensation cells). It is clear that it is not advisable to use for these structures, a name which suggests a function until their function has been ascertained. So, for the sake of convenience, I shall use the term "protuberance" inasmuch as it does not suggest a function.

In the present work I shall confine myself to these protuberances in Necturus.

Review of Literature.

Hoffman (1878) was probably the first writer to mention these protuberances in Necturus. I have not been able to see Hoffman's work but according to B. F. Kingsbury, Hoffman described these

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protuberances in Necturus as glands and "speaks of their almost circular opening upon the surface epithelium of the intestine."

Oppel in 1889 saw protuberances in the intestinal submucosa in Proteus anguineus and pronounced them glands (Drüsen). He noted that mitotic cells were abundant in the structures and in the intestinal mucosa in the immediate vicinity of the structures.

Protuberances in the intestinal mucosa of Triton were described in 1892 by Bizzozero. He referred to them as groups of compensation cells (Ersatzzellen) and sprouts (Sprossen). He said that cells proliferate not only at the base of the mucosa but press the wall of the mucosa out in places to form sprouts. For saying that these sprouts are epithelial, he gave the following reasons, which are summarized from the translation; (1) their general character and constitution are those of epithelial tissue, (2) cells can be seen in all stages of transformation from the cubical cells of the sprout to the columnar cells of the mucosa. He remarked that mucous granules can be seen between the epithelial elements and that a great many mitoses are present in the sprouts.

In Salamander protuberances have been described by Nicholas (1894) who called them proliferation buds (burgeons germinatifs). Nicholas said that they represent cell proliferation centers, and that they could not be glands inasmuch as they possess no lumen. He said that dividing cells were to be seen only in the buds (bourgeons) or in the surface epithelium near the neck of a bud.

Kingsbury in 1894 working on the enteron of Necturus regarded these protuberances as glands. He wrote "my study of them in Necturus would, however, lead me to regard them as glands. The arrangement of the cells as if surrounding a lumen, and indeed a lumen itself, which Nicholas declared did not exist in these structures, could be seen upon almost all of my sections in some of the glands. I was unable, however, quite satisfactorily to demonstrate the existence of a neck, although it seemed in numerous glands to be quite well indicated."

The work of Bizzozero was resumed and confirmed by Sacerdotti in 1896. The latter also working on Triton sought to ascertain the relation between the period of cell division and the period of mucous secreting activity. He determined that karyo-

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kinetic forms were assumed by cells which had already begun to secrete mucous, that these karyokinetic cells are found in the deeper parts of the epithelial layer but more especially in the epithelial spigots (Zapfen) pressing into the connective tissue. He added that occasionally and exceptionally karyokinetic cells were seen near the surface of the epithelium. Sacerdotti explained their presence in that region by saying that the cells in the animal concerned were possessed of very great growth capacity and thus certain mitotic cells had been forced out of the epithelial spigots and had reached the surface while still in mitosis.

Bates in 1904 described protuberances in the American Amblystoma and remarked that their structure would suggest glandular activity yet said he was not able to demonstrate the existence of a lumen.

Technique.

Inasmuch as the main object of my work was to determine the finer structure of these protuberances and with a view to distinguish whether they were glands or not, I endeavored to obtain a technique which would give to glandular secretions a differential coloration. For this purpose some of Dr. R. R. Bensley's special methods for glands were used.

Mallory's triple stain (Guyer, Animal Microcology, p. 172) was used extensively. It proved to be especially rapid and practical for general work. It gave a conspicuous differential coloration to connective tissue and epithelial tissue.

Weigert's Hematoxylin (Guyer, p. 178) proved to be very effective for preparing slides for the purpose of studying mitosis and details of nuclear structure.

Some of the animals were killed very soon after being brought into the laboratory, others were kept in an aquarium for a few months. Most of the fixing was done in Gilson's fluid but some fixing was done in Bensley's Acetic Osmic Bichromate fluid.

Description.

These protuberances in Necturus are located for the most part at the peripheral or outer parts of the folds. Occasionally one is found on the side of a fold a short distance from the peripheral bend. In one animal, however, out of over twelve which were

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studied, in which the intestinal mucosa was more abundant and more folded, I saw a few of these protuberances occupying positions at the very central part of the sections at the central apices of some of the folds.

As for the distribution of these protuberances throughout the length of the intestine, I found that they are more abundant anteriorly than they are posteriorly. To ascertain this, I made a count of the number of protuberances that appeared in cross sections in the respective regions. The count involved twenty slides from each region from about five different animals. The results of the count are here summarized.

Region	Maximum No.	Minimum No.	Average No.
Duodenal region	109	43	70.3
Middle intestine	104	45	66.6
Rectum	75	37	52.6

Thus it is seen that the number of protuberances decreases with distance from the pylorus.

The size of these protuberances varies in diameter from about 50 micra to about 127 micra. In length they vary from about 127 micra to about 293 micra. These measurements made with a stage micrometer and an ocular micrometer, are the extreme measurements of a great many taken.

The shape of these protuberances is naturally variable but it is usually possible to distinguish three parts. (1) the body, that part of the structure which lies wholly outside the mucosa, (2) the mucosal portion, or the portion that lies wholly within the mucosa and (3) the neck which connects the body and the mucosal portion. I shall describe each part separately and in detail.

The body is commonly roughly spherical in shape but is also frequently columnar or conical with the point more or less blunt. The body is also frequently branched. These structures are invariably connected to the mucosa. In mounted sections one sees many bodies apparently not connected to the mucosa but by tracing such bodies through serial sections a connection with the mucosa could always be demonstrated.

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Those protuberances that are situated quite at the peripheral ends of the folds almost always extend into the submucosa in a direction perpendicular to the mucosa. If however the protuberances are situated on the sides of the folds, as related above, the body part bends over so as to lie close to the mucosa. In other words, the body parts of the protuberances always extend in a direction radiating from the center of the intestine.

The cells in the body portions are polygonal in shape. The nuclei are more or less spherical and occupy nearly all of the cell. I studied carefully the narrow zone of protoplasm which surrounds the nucleus in cross sections to see if I could discover any granules the presence of which would suggest a secretary activity, but none of my preparations showed any.

I paid particular attention to the arrangement of cells within the body, and was unable to demonstrate a universal definite order of arrangement. This is contrary to the findings of Kingsbury, who stated that the cells were arranged as if surrounding a lumen, and added that a lumen itself could be seen in some of his sections. I was unable to discover a lumen nor did I find an arrangement of cells that would suggest the presence of a lumen.

Therefore, I have concluded that these protuberances cannot be glands. I consider the presence or absence of a lumen a sufficient criterion on which to base such a conclusion, for if these structures were glands the existence of a duct through which the secretions might pass and a corresponding arrangement of cells would be necessary.

The mucosal portion is that portion which extends into the epithelial mucosa. It commonly spreads out within the mucosa so that cross sections appear fan shaped. There is a gradual transition in the shape of the cells of the body of the protuberance and the columnar cells of the mucosa. The transition takes place in the mucosal portion of the protuberance. The cells in the mucosal portion of the protuberance become more and more elongate and are arranged in crescentic layers in optical section, as if suspended hammock-like from the outer boundary of the mucosa. The farther the cells are from the center of the protuberance, the more columnar they are. The nuclei become more and more oval.

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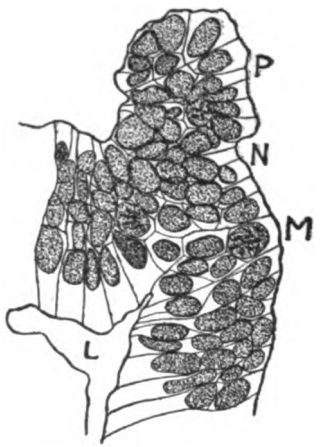
The neck of the protuberance is the portion at the peripheral boundary of the mucosa, which connects the body of the protuberance to the mucosal portion. It is almost always somewhat constricted.

Cells in various stages of mitosis are frequent in all parts of the protuberance. To determine the ratio of mitoses present to the total number of cells in the protuberances, I made a count involving several thousands of cells and calculated that the ratio of mitotic cells to the total number of cells is one to forty-one. There are an average number of 39 cells in a section of a protuberance. Thus there is an average of about one mitotic cell in a protuberance. In some protuberances I saw three cells in mitosis while, on the other hand, there were many sections of protuberances that did not show a single mitosis.



Camera lucida drawing of a portion of a cross section of the mucosa of the mid intestine of Necturus maculatus showing protuberance P, lumen L, neck N, mitotic cells M. The cell walls were not drawn by means of a camera lucida. Spencer Ob. 10X, Oc. 4.

It is significant that not one case of mitosis was seen in the mucosa at any considerable distance from a protuberance.

Upon these facts I have concluded that cells which are to compose the intestinal mucosa in Necturus, are formed in these protuberances. It would seem that the cells are forced through the neck of the protuberance, while perhaps sometimes still in mitotic condition. In the mucosal portion of the protuberance, the cells appear to be forced in a lateral direction along the mucosa. Since mitotic cells are not found at points along the mucosa excepting near the protuberances, it may be concluded that the intestinal mucosal cells do not divide after they have become functional cells.

According to this view, these protuberances in Necturus are cell proliferation centers for the mucosa as was concluded for some other Urodeles by Bizzozero and Nicholas.

Mitchell, South Dakota.

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FILICOLLIS BOTULUS N. SP., WITH NOTES ON THE CHARACTERISTICS OF THE GENUS*

By H. J. Van Cleave

Lühe[1] founded the genus Filicollis (1911:30) upon the characteristics of the species formerly known as *Echinorhynchus anatis* Schrank, of which he considered *E. filicollis,* Rud., *E. polymorphus* Bremser, and *E. lævis* vonLinst. as synonyms. In this species, *F. anatis (Schr.),* the female has a peculiarly modified proboscis at the end of a slender neck. Only a portion of the surface of the large inflated proboscis carries hooks. This characteristic was incorporated into Lühe's definition of the genus. Regarding the development of the proboscis in *Echinorhynchus filicollis* Rud., which as indicated above Lühe accepted as a synonym for *Folicollis anatis,* de Marval[2] (1905:267) has given a complete account of the changes in form accompanying advance in age of the female. His figures 103, 104, and 106 indicate in the development of the female a gradual change from an ovoid proboscis slightly larger than the neck in very young forms to the inflated, spherical form characteristic of the fully mature female. These three figures have been copied by the writer in the present article as figures 1, 2, and 3. In order to evaluate this point of structure fairly it should be borne in mind that these changes in the form of the proboscis occur after the individual has found lodging in the final host. To the writer it seems within the bounds of reason that such a characteristic as the shape of the proboscis appearing near the end of the development of the individual carries with it but slight phylogenetic significance. Consequently it could signify nothing more than a specific character and no longer should be considered as diagnostic for the genus.

The writer has found individuals belonging to a new species which agree in all essential details with the description of the genus Filicollis except in the lack of this inflated proboscis of the female. The creation of a new genus for such a minor variation would necessitate the separation of forms which are evidently closely related.

*Contributions from the Zoological Laboratory of the University of Illinois, No. 63.

[1]Lühe, M. 1911. Die Süsswasserfauna Deutschlands, Heft 16, Acanthocephalen. Jena; 116 pp.

[2]Marval, L. de 1905. Monographie des acanthocéphales d'oiseaux. Rev. suisse de Zool., 13:195-387.

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To avoid this confusion the description of the genus should be modified so that the species *F. botulus,* described later in this article, may be included within its limits. In view of the fact that this adds but one more species to the genus the writer does not feel justified in offering a complete emendation of Lühe's definition but offers as a suggestion that until more is known of members of this genus the original definition (Lühe 1911:30) should be qualified by adding the statement: "The proboscis of the female *may* be dilated, or in some species the proboscis of the female may assume the same form as that described for the males of the genus."

Filicollis botulus n. sp.

Description. Body both sexes cylindrical, large, thick, sausage shape; about 20 mm. long; about 4 mm. in diameter. Neck long, naked, retractable; 0.76 mm. long; 0.38 mm. diameter at posterior end. Proboscis sheath 2 mm. long. Body of male spined short distance back from neck, spines about 0.012 mm. long. Mature female with no distinct spines in this region, cuticula of anterior part of body in minute elevations. Proboscis ovoid, 0.65 mm. long, 0.57 mm. in diameter, armed with sixteen longitudinal rows of seven or eight hooks each. Hooks practically uniform in size, basal hooks 0.060 to 0.062 mm. long, apical hooks slender, basal hooks without distinct roots. Embryos elliptical, with three concentric membranes; 0.071 to 0.083 mm. long, 0.030 mm. broad. Type host *Somateria dresseri,* in intestine. Type locality Maine, U. S. A. Cotypes in collection of Bureau of Animal Industry, Washington, D. C., catalog number 2080; and in collection of the writer at Urbana, Illinois.

The material upon which this specific description is based was collected by Mr. Albert Hassall in Maine during the month of April, 1892. There are fifty specimens in the collection examined by the writer. In addition to this material the collections of the Bureau of Animal Industry contain several hundred specimens of this species from the type host and from *S. mollissima* [*S. m. borealis?*]

Acanthocephala of this general form from American ducks of various species frequently have been assigned by earlier workers to the species *'Echinorhynchus polymorphus.'* The confusion of

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the species *E. polymorphus* Bremser, *E. anatis* Schrank, and *E. filicollis* Rudolphi of the early workers has been very general. After various investigators had attempted to solve the problem of the relationships of these species Lühe (1911; 27 and 30) has finally made a careful analysis of the characteristics of these species through which he was led to establish two independent genera, Filicollis and Polymorphus. The addition of another species to the genus Filicollis furnishes support to the contention for the validity of this genus, which is very clearly a natural group in the classification of the Acanthocephala.

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EXPLANATION OF FIGURES

All original figures were drawn with the aid of a camera lucida. A projected scale indicating the magnification accompanies each drawing.

Fig. 1. *Filicollis anatis,* fully mature female redrawn from deMarval 1905, fig. 103.

Fig. 2. *F. anatis,* young form. Redrawn from deMarval 1905; fig. 104.

Fig. 3. *F. anatis,* very young form. Redrawn from deMarval 1905; fig. 106.

Fig. 4. *F. botulus* n. sp., male. Neck retracted. Body spines not shown. Arrow indicates posterior limit of spined region.

Fig. 5. Cuticular body spines from anterior region of body of male shown in fig. 4.

Fig. 6. *F. botulus* n. sp., female. Tip of proboscis slightly inturned.

Fig. 7. *F. botulus.* Proboscis and part of neck of female.

Fig. 8. Profile, ventral surface, of proboscis shown in fig. 7.

Fig. 9. *F. botulus.* Embryos from body cavity of female.

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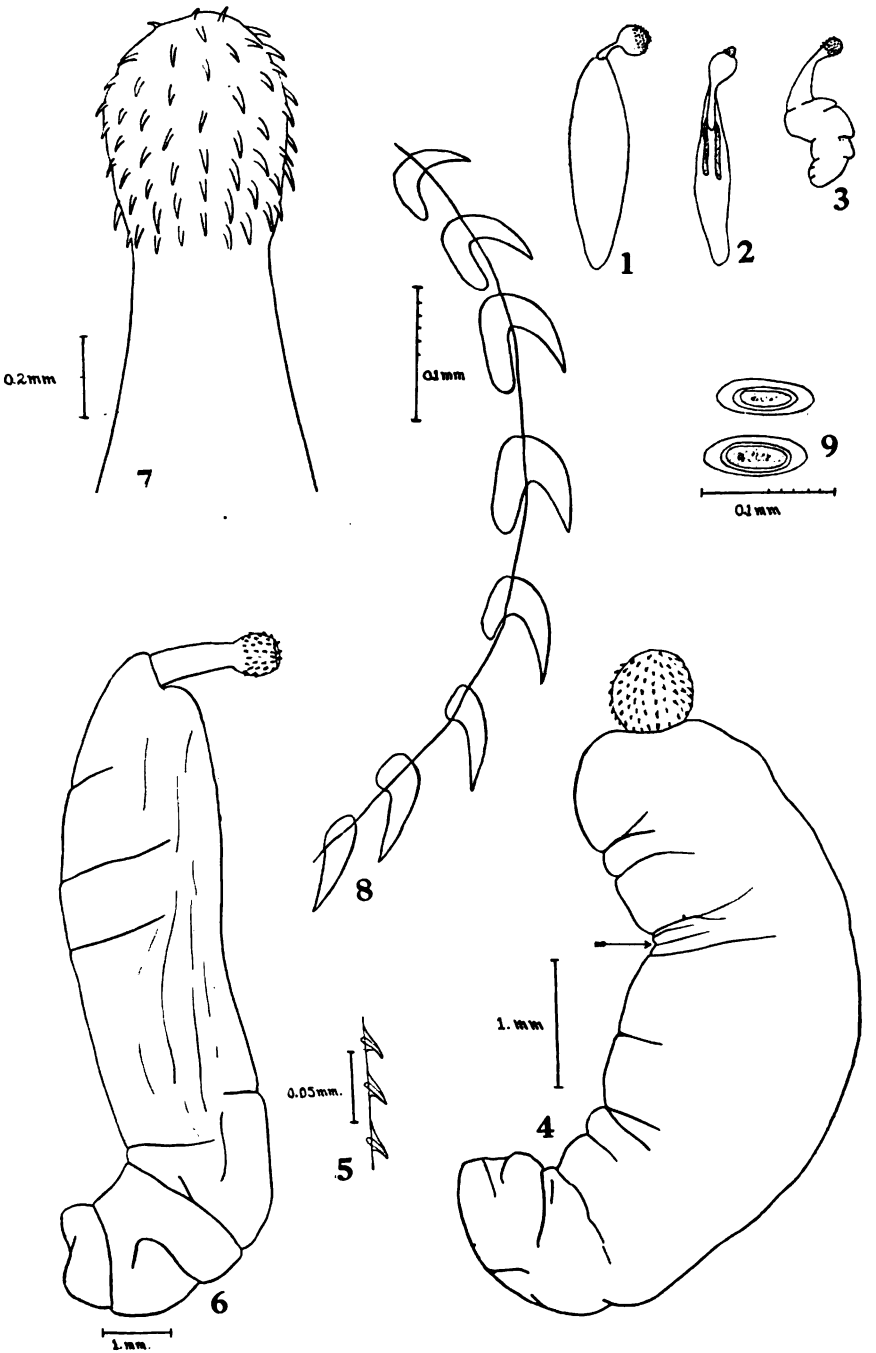

PLATE XVIII

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated suggestions of suitable fields of investigation.—[Editor.]

NOTES ON HANDLING PROTOZOA IN PURE LINE WORK

During the past year the writer has been engaged in experiments on the inheritance of extra contractile vacuoles in a new race of Paramœcium and has worked out some methods of technique that have so facilitated his work that he is led to publish them in hope that they may be of benefit to others.

Maintaining pure cultures.—The greatest care is necessary to prevent pure line cultures from becoming mixed with others. Even with labelled pipettes accidents may occur. The scheme shown in

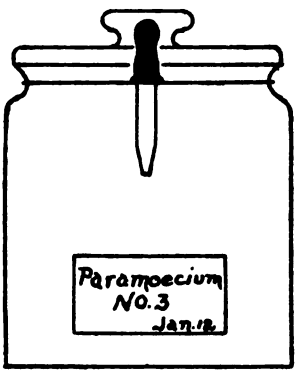

FIG. 1

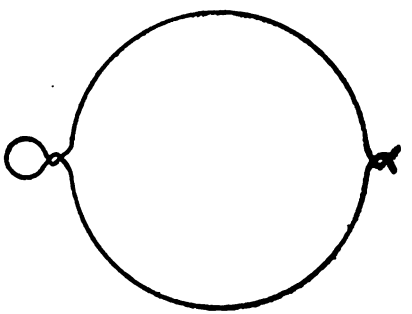

FIG. 2

the cut was recently devised and has proved most convenient. A piece of soft brass wire is shaped about some round object of a diameter slightly larger than a pipette and is held by several twists. Then the long ends of the wire are bent around the culture jar and again fastened by twisting the ends. In the jars used by the writer (humidors bought at any five and ten cent store) there is a convenient groove near the top into which the wire fits nicely. When finished the small circle protrudes from the jar and into

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this ring the pipette is dropped giving the appearance of Fig. 1. With this method pipettes are always at hand and there is no danger of mixing the lines by transfering animals (clinging to the walls of a pipette) from one culture to another.

Preparation of watch glasses.—Syracuse watch glasses have been used for single individuals throughout the writer's work and considerable difficulty was experienced at first in locating animals which were close to the edge of the container. They frequently found their way there as the fluid had a tendency to spread evenly over the surface of the watch glass. Several methods were tried to correct this tendency of the culture medium to spread over the bottom but the best one was hit upon accidentally. There was a trace of paraffin in a pan in which the glasses were being sterilized one day and this coated the glasses imperciptibly but sufficiently to give the liquid no hold on the glass. In vessels treated in this way the surface tension of the medium tends to draw it into a spherical mass. Should the liquid roll to the edge of the glass where the animals would be hidden from view, it is easily rolled out again by tilting the glass and none of the animals in the drop are left behind. When animals are being kept in very small drops of water the writer has placed as many as twenty individual drops of liquid containing protozoa in a single watch glass and they have not run together. The surface tension of the liquid draws it up, when on a paraffined surface, until it gives a very fair picture of a drop of mercury. Furthermore, being contracted to the smallest area possible there is less evaporation than when the same amount of fluid is spread out and the chances of losing a valuable specimen through drying are very much less. The writer's practice is to use a piece of paraffin about the size of a pea to a quart of water. This will be sufficient for a surprising number of watch glasses. When the sterilized glasses are removed they are wiped while hot and polished. No paraffin is visible although a faint trace of it can be felt.

Making Pipettes.—In this work the most useful pipette has been found to be one that has a short but very fine tip. The usual methods of drawing them tend to make a pipette with a rather long tip as the glass tube has been heated for some distance

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equally along its length. To cut down the area heated the tube to be drawn is placed transversely across a fish tail flame, which heats equally an area certainly not more than a quarter of an inch at found their way there as the fluid had a tendency to spread evenly the most, and the tube is pulled with considerable force when the glass is just commencing to melt. Several trials will show the best time to start pulling. This method gives pipettes with very fine tips not more than from three-quarters to one and one-half inches long.

Zoological Laboratory, ROBERT T. HANCE.
University of Pennsylvania.

NOTES ON EMBEDDING IN PARAFFIN

When embedding very small objects, such as insect larvæ or small flowers or anthers, in paraffin it is most convenient to orient them one behind the other. This method allows a single block to be made of from three or four to a dozen pieces of tissue and these may be cut in one ribbon. This obviously eliminates a great deal of the labor involved in making a block of each separate object, cementing it to the holder, trimming it and adjusting the microtome each time. In the ribbon it is easy to see where one piece of tissue ends and the other begins as there is usually several blank sections of paraffin between them. It is relatively simple to arrange the tissue in line under a carbon bulb with warm needles but a difficulty is met with when an attempt is made to place the paraffin mold in water for cooling. The material is shaken from position and must be reoriented. This had been overcome in the following way. A watch glass is used as a mold for embedding small objects and a petri dish is convenient for larger tissue. When the tissue is ready to be embedded the dish is heated to the melting point of the paraffin under the electric bulb. It is then placed in a crystallization dish with two slides beneath it to prevent it from touching the bottom of the container. Paraffin is then poured into the small dish and the objects oriented as desired the heat of the electric bulb keeping the paraffin melted. Then the light is turned off and cold water is poured into the crystallization dish. Since the dish containing the paraffin is raised from the bottom the water flows under it and soon solidifies the paraffin in the lower part of

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the dish which consequently holds the objects fast. As soon as a surface film is formed enough water can be added to cover the embedding mold to complete the hardening of the paraffin.

In petri dishes or watch glasses the bottom is practically flat and true and the tissue is allowed to sink to the bottom. When the tissue is cut out as a block the part that rested against the bottom makes one of the two parallel sides and requires little or no trimming.

When a number of pieces of tissue or a number of series (as described above) are embedded in one disk of paraffin it is dangerous to attempt to separate them with a knife as one can never be sure of the direction the crack in the paraffin will take. I have found that a hand scroll saw or coping saw (which may be purchased for ten to twenty-five cents) does admirably for cutting a block of tissue from the main disk. A hot wire is used by some but is not nearly so convenient nor so accurate as the saw. The use of the saw permits many more pieces to be placed in the same space as no care need be taken to have well defined pathways for the paraffin to split along as is necessary when a knife is used for separating the pieces.

ROBERT T. HANCE.

A NEW SPECIES OF OPERCULARIA

Opercularia wallgreni Grier n. sp.

Plate XIX. Figs. 1 and 2

Bodies ovate or attenuate fusiform, about 3 times as long as broad, tapering mostly toward the pedicle extremity. Ciliary disc never elevated above the margin of the peristome a greater distance than ½ the length of the animal, apparently with but one circlet of cilia. Membranous collar moderately large, but obliquely set. Endoplast band-like, curved, parenchyma beneath granulated. Pedicle tree-like, slender, branching profusely and dichotomously, attaining a considerable proportionate altitude, delicately striate in a longitudinal direction. Transverse articulations wanting or present only where branching occurs.

Height entire polypidum 1.4 mm., length extended zooid .10 mm., width .022 mm., width of pedicle .005 mm.

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Fig. 1.

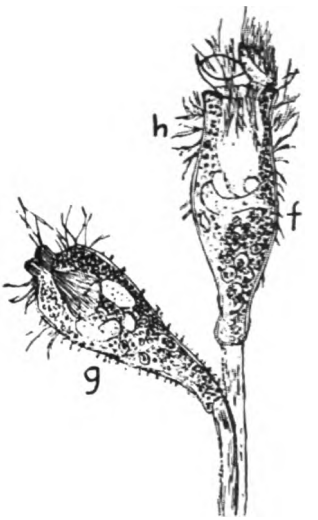

Fig. 2

PLATE XIX

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Habitat.—Fresh water, apparently only upon aquatic plants. The colonies may include from 2 to about 200 zooids, which assume a nodding or pendant position after contracting. The species described was found in an aquarium in the writer's laboratory at the Central High School, first growing upon *Sagittaria platyphylla* Smith, but readily attaching itself to *Elodea, Myriophyllum,* and other aquatic plants. Its food consists for the most part of unicellular Algæ although Protozoa were sometimes observed in process of digestion. The species is very prolific, and while it does not grow in a hay infusion, quickly covers the walls of aquaria, growing thickest on the sides nearest the light. It is apparently of great longevity. This form I have respectfully dedicated to Dr. A. B. Wallgren, Professor of Zoology, University of Pittsburgh.

EXPLANATION OF FIGURES

PL. XIX.

Fig. 1. Entire colony x about 90.
a, b, c, d. Successive positions assumed by zooids during acquisition of food.
e. Curious introverted position noted.

Fig. 2. Two zooids x about 400.
f. Expanded, taking in food.
g. Contracted.
h. Attached bacterial growth.

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N. M. GRIER.

A METHOD OF MAKING TOTO MOUNTS OF UNICELLULAR FORMS

The matter of making toto mounts of unicellular forms often presents considerable difficulty. The cells or cœnobia settle so slowly that there is danger of losing them in the changes of liquid, and this slowness in settling makes the use of the more precise stains difficult. A method which has been successfully used for small forms like *Scenedesmus* is described in Chamberlain's "Methods in Plant Histology," University of Chicago Press, 1915. It consists of drying the cells down on the slide and then carrying them through all subsequent processes on the slide as in the case of paraffin sections. This method seems to cause some distortion

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even in the smaller forms and a large form like *Closterium* is ruined. The following method, discovered in the botanical laboratories at the University of Nebraska, has been found to combine the good fixation and preservation of the bulk method with the precision of staining and the ease of handling secured by drying the cells to the slide.

The material is killed and fixed in whatever solution the investigator has found most satisfactory for the particular group of algæ or Protozoa with which he is working. It is washed in bulk in the usual manner and carried through a graded series of alcohols until a strength of about sixty per cent. is reached. It is allowed to settle completely in this grade. A very thin layer of albumen fixative is smeared upon the thoroughly cleaned slides. A drop of the material is then drawn up with a pipette and placed upon the slide. The sixty per cent. alcohol in which it is lying coagulates the albumen and causes a surprisingly large number of cells to be firmly fixed to the slide. They may now be dipped directly into sixty per cent. alcohol and successively into higher grades. It is possible to use such stains as Flemming's triple and iron-alum hæmatoxlin rapidly and with precision. Before using a stain like Flemming's triple it is usually well to harden the cells thoroughly in ninety-five per cent alcohol, and then proceed as usual.

Univ. of Nebraska. ROBERT A. NESBIT.

METHOD TO CLEAN USED MICROSCOPIC SLIDES

Especially where a course is given in microscopic technic there are usually a large number of worthless slides prepared. To throw them away seems an extravagance and yet to clean them in waste-xylol is practically a waste of time.

The method I am about to suggest may be well known, yet I think it will bear repeating. There had been a large number of old slides collecting from year to year in our department, worthless and merely occupying space, yet no one cared to assume the responsibility of throwing them away. Recently Professor Reese head of the department, suggested we try gold dust in an attempt to clean them.

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A liberal amount of gold dust and a number of the slides, some of them dated 1902, were placed in water, and thoroughly boiled. As soon as the cover slips came off of their own accord, the slides and slips were placed in a pan of water. These were wiped dry while others were being boiled. The ease with which they can be cleaned and dried and the small amount of time required compared to the waste-xylol method, makes it a very profitable undertaking.

On taking them from the gold dust solution they were first placed in waste alcohol, but it was found by placing them in water they could be cleaned and dried much easier.

Zoological Laboratory, J. THERON ILLICK.
West Virginia University.

ENTOMOLOGICAL NOTES

Chromosomes of Notonecta.—Browne ('16, Journ. Morph., 27:119-162) has made a comparative study of the chromosomes of five North American species of *Notonecta (undulata, irrorata, insulata, shooterii,* and *indica)* and one species of the same genus from Europe *(glauca)*. Among other things, it was found that an XY pair of chromosomes is present in each of the above-mentioned species, the components of which divide separately in the first division and go to the opposite poles in the second. The X and Y chromosomes vary in size in the cells of the different species as well as in the cells of different individuals of the same species. They are almost equal in most of the cells of certain individuals of *shooterii,* while in *indica* they are distinctly unequal. *Undulata, indica,* and *shooterii* have 14 chromosomes in the first division, 13 in the second, and 26 in the diploid groups. *Irrorata* and *glauca* have 13 chromosomes in the first division, 12 in the second, and 24 in the diploid groups. *Insulata* has 14 or 13 chromosomes in the first division and 12 in the second. Large double chromosomes occur in *insulata, glauca,* and *indica.* No definite correlation of the somatic characters of the different species with the difference in chromosomes number and arrangement was discovered, although it was found that the 14-chromosome species are the smaller and the 13-chromosome ones are the larger. It thus appears that while,

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in general, a definite number and arrangement of the chromosomes of each species exist, the status of each species in relation to the others cannot at present be determined on this basis. The chromosomes and somatic characters seem to indicate that *indica* has been derived from *undulata*.

Mitochondria.—Lewis and Robertson ('16, Biol. Bull., 30:99-124) studied, by the tissue culture method, the mitochondria and certain other structures in the male cells of the grasshopper *Chorthippus curtipennis*. By using a culture medium which very closely approached Locke's solution, it was found that, in addition to the fact that the minute structures of the living cells could be examined from day to day, these structures could be experimented upon as readily as those of the chick embryo. Any stage in the development of the germ cell was obtained by this method and was studied in the stained and unstained conditions, the staining being done with Janus green and neutral red. By these methods, mitochondria and neutral red granules were demonstrated in the primary spermatogonium. The former are present in the primary spermatogonium as small, delicate granules and increase in quantity during the growth stage, becoming definitely arranged along the spindle during the spermatocyte division. In the spermatid, they form the nebenkern, later developing "into two equal homogeneous threads in the tail of the spermatozoön."

Gynandromorphism.—Cockayne ('15, Journ. Genetics, 5:75-131), in a paper entitled "Gynandromorphism and Kindred Problems", presents a comprehensive discussion of gynandromorphism among animals, the greater part of the data being drawn from insects. Descriptions of a number of new examples are presented. The data included in the paper are not readily summarized and cannot be included here. A somewhat elaborate and suggestive classification of "hermaphrodites" is given. The theoretical explanations of gynandromorphism are taken up in some detail and considered critically. A number of text figures illustrate the condition of the internal reproductive organs in some of these anamalous insects. Four plates devoted exclusively to Lepidoptera illustrate certain forms of gynandromorphs. A bibliography of sixty-eight titles accompanies the paper.

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Color Changes in Dynastes.—Andrews ('16, Journ. Exp. Zool., 20:435-456) reports results of studies on color changes in adults of the rhinoceros beetle, *Dynastes tityrus*. It was discovered that when live specimens of either sex which were light yellow with dark spots were confined for a time in receptacles with wet decayed wood, they all became very dark reddish with the spots scarcely visible. When removed from these conditions, they rapidly returned to the usual light coloration. Since such changes in an animal having no changeable pigment cells or blood vessels so distributed as to make color change possible seemed to be unrecorded, experiments were undertaken to determine the nature of this phenomenon. Variations in moisture were found to underlie the conditions which produced the color changes. These color differences can be explained without the assumption of internal nervous activity since, in general, the reactions of a dead, dried specimen were the same as those of the living beetle in all respects concerning the change of color from light to dark and the reverse. Experiments with light, heat, and moisture yielded results which made it evident that both living and dead beetles behaved alike in changing color under conditions which were interpreted as chiefly involving differences in the amount of water presented to the surfaces of the elytra and the thorax. Results of experiments pointed to the conclusion that any liquid which can enter the shell may cause it to change from light to dark color. Microscopical examination of the elytron showed that it is composed of an outermost layer of such a nature that it readily absorbs and gives off moisture. The absorption of liquids permits the color of the underlying part of the exoskeleton to appear as dark red, whereas when air replaces the moisture in this layer, it prevents the underlying color from showing through. The dark spots which do not change appear to be due to the presence of some material which so fills the pores or interstices that it acts as if it makes the area permanently wet or to some degree soaked with liquid, thus rendering visible the underlying color. The relation of these color changes to the life activities of the insects is not known.

Light Reactions of Vanessa antiopa.—Dolley ('16, Journ. Exp. Zool., 20:356-420) finds that *Vanessa antiopa* is invariably

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positively phototactic and in direct sunlight comes to rest with the head away from the source of light. Specimens with one eye blackened and placed face foremost in a horizontal beam usually turn toward the functional eye, occasionally continuing to turn in this fashion, thus performing circus movements, but usually reversing the movement at the edge of the beam and moving toward the source of light. In non-directive light, the insects perform circus movements only, each turning toward the functional eye. Apparently, the stronger the light the larger are the circles described by the insect. Non-directive light of very low intensity does not deflect either way specimens with one functional eye but a further reduction of this light leads to the performance of circus movements toward the blinded eye. The behavior becomes modified by repeated trials, the modification being manifested in three ways: (1) decrease in number of circus movements; (2) decrease in angle of deflection; and (3) increase in promptness of orientation at edge of beam. With one eye blackened, this insect, when moving toward a source of light, can re-orient when the direction of the rays is changed and always in such a way as to turn toward the source of light. In darkness, blinded specimens move in circles toward the blinded eyes, showing that the covering acts as a stimulus. In light, blinded specimens circle in the opposite direction, showing that light received by the functional eyes is the stimulus. Evidence indicates that the reaction may depend upon the localization of photic changes within the eyes and that orientation is not wholly dependent upon the relative intensity of light on the functional and blinded eyes.

Origin of Wings.—Crampton ('16, Journ. N. Y. Ent. Soc., 24:1-39) presents a thoroughgoing summary and critical examination of the various theories relating to the origin of wings in insects. Special attention is given to a comparison of the evidences advanced in support of the *tracheal gill theory* and the *paranotal theory* of the origin of insect wings. The latter is favored and some original data are offered in its support. The wings of all insects are regarded as homologous and of common origin, thus being subject to the same principles regardless of the kind of metamorphosis. Tracheal gills and wings have been shown by em-

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bryological studies to belong to different developmental series and are not homologous, facts which offer very strong counter-evidence against the derivation of wings from tracheal gills. Since the paranota (integumental outgrowths on the sides of the tergum) are homodynamous with the wings, the latter "were doubtless derived from them, since they occur in the most diverse forms". The only reliable evidence available at present is that of embryology, such evidence indicating that the wings are of tergal origin. It is also concluded that the paranota from which the wings are thought to have originally developed were entirely or in part expansions of the tergum. A bibliography of two hundred twenty-two titles accompanies the paper.

Wing Venation of Hymenoptera.—Rohwer and Gahan ('16, Proc. Ent. Soc. Wash., 18:20-72) have published a paper on the "Horismology of the Hymenopterous Wing" in which a modified form of the old Cresson system of wing venation terminology is proposed. The paper is valuable because of the extensive synonymy and comparisons of the systems used in the past, thus making much easier the translation of one system into another and the determination of equivalent names. The new system which is proposed is based on the opinion of the writers that "it is better for taxonomatic work to designate a given area by a given name and call it that regardless of its possible homologies or analogies." The new system is constructed only for insects of the order Hymenoptera and is apparently a revival of the old custom of using different systems of terminology for venation in the different orders and of using purely arbitrary systems rather than those based on homology. The writers point out what they consider to be objections to the Comstock-Needham system but there is doubt that these objections are well taken. Unfortunately, such a system as the one proposed in this paper, in spite of its possible commendable points, will have the effect, if adopted at all extensively, of impeding the development of a common system, based upon homologies, for all of the orders of insects, a system much to be desired.

Parasites.—Timberlake ('16, Can. Ent., 48:89-91) finds that certain insects (beetles) may be parasitized by *Dinocampus americanus,* a common braconid parasite, without producing the death

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of the host. It was observed that certain individuals of *Hippodamia convergens, Coccinella 9-notata,* and *Olla abdominalis* might be parasitized, the larva attain full growth and escape, and the recovery of the host be apparently complete. Experiments in which individuals of *Hippodamia convergens* and *Olla abdominalis* were exposed to the parasite showed that this particular parasite does not injure the vital organs of the host, although its fatty lymph tissues are often left in such a depleted condition that the beetle soon dies. The exit aperture of the parasites is itself sometimes fatal in effect. These experiments showed that successive parasitism may occur, resulting in the emergence of more than one generation of the parasite from the same host.

Insects and Fire Blight.—Stewart and Leonard ('16, Phytopathology, 6:152-158) report the results of experimental studies on the rôle of insects in the dissemination of fire blight bacteria. Experiments with certain Diptera *(Pollenia rudis* and *Sapromyza bispina)* seem to indicate they they are not active agents in increasing the number of twig blight infections, since their method of feeding is such that it is doubtful if the blight bacteria which they may carry can gain entrance to the tissues. Possibly the bacteria carried by the flies may gain entrance through the punctures of other insects and produce a few infections. Flies are thought to be most important in those cases where they carry the causal organism to blossoms and occasionally to wounds, such as those produced by hail stones. The authors believe that all of the sucking bugs in nurseries are important in this connection. The numbers, methods of feeding, and seasonal distribution make the various species of different interest and importance in the transmission of blight bacteria. It is suspected that those which feed upon the tender tips of the twigs are of more importance than those which feed upon other parts of the foliage. *Lygus invitus, Heterocordylus malinus* and *Lygidea mendax* are of importance in spreading fruit blight in orchard trees.

Embryology of Honey-bee.—Nelson ('15, Princeton Univ. Press) has recently published a book on "The Embryology of the Honey Bee", which is the most comprehensive and thoroughgoing treatise that has yet appeared on this subject. The author has been

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concerned not only with the details of the development of the honey-bee but he has employed the comparative method of treatment, drawing extensively upon the works of other investigators on the embryology of insects in general, thus making the book valuable in connection with any work in insect embryology. The large mass of data cannot be summarized here but certain special features deserve mention. The duration and rate of development were specially considered and it was found that the total time normally required for embryonic development is 76 hours. This period is divided into stages I-XV. Cleavage requires 14-16 hours; formation of blastoderm, 14-16 hours; formation of mesoderm, rudiments of mesenteron and embryonic envelope, 12-14 hours; remainder of development, including differentiation of tissues and organs, 32-34 hours. Discussion of the formation of the mesenteron is accompanied by an extensive comparison of the various opposing views and interpretations of other investigators on this subject. The different writers are classed according to the following theories of mesenteron origin: (1) derivation from yolk cells; (2) derivation from the lower layer (mesoderm, entomesoderm, primary entoderm); (3) derivation from proliferations of the blind inner ends of the stomodæal and proctodæal invaginations; (4) derivation, independent of the mesoderm, from two proliferating areas of the blastoderm, one at each end of the germ band, corresponding to the future location of the stomodæum and proctodæum, respectively; and (5) derivation from cells migrating inward from thickenings or islands of the blastoderm. Nelson holds that "the relation of the mesenteron rudiments in the honey bee may be interpreted in two ways, and the one chosen will probably depend largely on the theoretical bias of the interpreter. First, the mesenteron rudiments may be referred to the mesoderm .....Second, the mesenteron rudiments may be considered, ...as purely blastodermal in origin." A final decision between these two interpretations is not attempted. Segmentation is considered rather fully and is found to be as follows: head, six segments; thorax, three segments; and abdomen, twelve segments. Appendages were observed on the antennal, the three gnathal, and the three thoracic segments. There is evidence that in the honey-bee the tritocerebral (intercalary) segment should be

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considered as "exaggerated ganglionic swellings" which probably do not represent appendages. Nelson has found nothing which could be "safely construed as abdominal appendages". Degenerating cells of unknown significance occur in the rudiments of the brain, particularly in the region between the second and third lobes of the protocerebrum. The tracheal system is formed from eleven pairs of invaginations of the lateral ectoderm, one pair, which seems to be observed for the first time, occurring on the second maxillary segment. This discovery is significant since "Now that a pair of tracheal sacs is known to exist in the second maxillary segment, the homology of the second pair of tentorial invaginations with the stigmata of the second maxillary segment is completely excluded, and the homology of similar invaginations with those of the trachea is decidedly problematical". The tentorium develops from two pairs of ectodermal invaginations, one in front of the bases of the mandibles and the other behind the bases of the first maxillæ. The œnocytes develop from the migration of cells from eight pairs of localized areas of the lateral ectoderm which occur on the first eight abdominal segments at the same level as the tracheal invaginations. The mesoderm differentiates laterally into a somatic and a splanchnic layer, the mid-ventral region remaining single layered. Separate cœlomic sacs are wanting. The single layered area develops into the rounded blood cells; the somatic layer forms the trunk muscles, the pericardial fat cells, and the dorsal diaphragm; and the splanchnic layer gives rise to the muscle layer of the mid-intestine and the two main divisions of the fat body. A mid-dorsal union of two rows of cardioblasts, derived from the two mesodermal layers, forms the heart. Two masses of mesodermal cells, one forming the anterior and one the posterior end of the mesoderm, produce the muscle layer of the stomodæum and the proctodæum respectively. One hundred fifty-seven titles are included in the bibliography at the end of the book.

Kansas State Agricultural College. PAUL S. WELCH.

NOTES ON OLIGOCHÆTA

Galvanic Response of Earthworm.—Moore and Kellogg ('16, Biol. Bull., 30:131-134) have tested the galvanic reaction of

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Lumbricus terrestris to constant current. Orientation began as soon as the current was submitted and the body of the animal took the form of a U with the concave side toward the kathode. Writhing movements accompanied this orientation and as a rule the animal ultimately crawled to the kathode. Pieces of the body of the worm, 3-4 cm. long, subjected to the constant current, responded in the same way, except that progressive movements were absent. Reversal of the current produced a reversal of the response. The reactions are interpreted as due to the tension of the longitudinal muscles on the kathode side of the worm, resulting in a stronger contraction than that which occurs in the anodal region.

South Indian Oligochæta.—Stephenson ('15, Memoirs of the Indian Museum, 6:35-108) reports results of studies on Oligochæta of Ceylon and Southern India. The eversible pharynx of certain enchytræids was studied and a sensory function is suggested for this organ. The constant presence of setæ within the cœlom of *Fridericia carmicheali,* surrounded by masses of lymphocytes, is discussed and the tentative explanation is that they are formed, not as are the setæ of the body-wall, but as excretory products which take the form of rods or spicules, persisting in the body-cavity or disintegrating and being eliminated through the usual channels. The so-called iridescent sperm funnels of some earthworms have been found to be due, not to iridescence of the funnel itself, but to the spermatozoa which are so disposed that they form innumerable extremely fine threads lying parallel and which function as a "diffraction grating". New species of the genera *Enchytræus, Fridericia, Drawida, Pontodrilus, Megascolides, Comarodrilus, Perionyx, Megascolex,* and *Erythræodrilus* are described.

Regeneration.—Hyman ('16, Journ. Exp. Zool., 20:99-163) has studied regeneration in a number of species of Oligochæta belonging to the families *Aeolosomatidæ, Naididæ, Lumbriculidæ,* and *Tubificidæ.* By the use of Child's cyanide method, a gradient in the rate of metabolism of two forms, primary and secondary, was demonstrated. In the former, the rate of metabolism decreases posteriorly from the head and was found only in *Aeolosoma* and the zooids of *Naididæ,* while the latter is superimposed upon the primary gradient but runs in the reverse direction. The secondary

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gradient involves the caudal third of the body in *Dero limosa*, the caudal half or more in *Lumbriculus inconstans*, and all but the first 5-15 somites in the *Tubificidæ*. Only the typical number of head somites are regenerated following amputation of varying numbers of anterior somites. The head regenerates a tail only when accompanied by a certain number of trunk somites and the tail regenerates a head only when it is of a certain minimum size whereby the gradient is eliminated. Under these conditions, regardless of length, a piece of *Dero limosa* regenerates a normal worm. If of appropriate length, any part of the body of *Lumbriculus inconstans* regenerates a normal worm, normal posterior regeneration occurring at any level. Formation of the head in *Tubifex* ceases at about the level of the fifteenth somite, while in *Limnodrilus* it ceases at the level of the seventh somite. The gradient of an axial series of pieces is not the same as that of the whole worm since temporary stimulation results from the cutting. Rate of metabolism is an important factor in anterior regeneration in short pieces since head formation will be inhibited in proportion to the metabolic rate of the old piece. If this rate be low, no inhibition of the new tissue occurs and a normal head is produced. Normal heads are always formed on long pieces since the dynamic factors are not important and the primary gradient determines the increased independence of cells at an anterior level over those of a more posterior level.

PAUL S. WELCH.

Kansas State Agricultural College.

NOTES ON THE COLLECTION AND REARING OF VOLVOX

Since the rearing of Volvox for laboratory use seems to be more or less unusual the writer has deemed it worth while to present here the results of some work done by a class of students and himself along this line.

Colonies of *Volvox aureus* Ehrenberg were found last fall in some abundance in small sphagnum pools on the west shore of a small glacial lake west of Ann Arbor known as the First Sister Lake. Collections were made on October 27, 1915. Small water-filled depressions in the sphagnum 10 to 30 inches in diameter were

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sought out and into these depressions pint jars were thrust in such a way as to secure some bits of sphagnum and decaying vegetable matter together with water. The contents of the jars were sedimented and the liquid just above the sediment examined for colonies. When found the water at the top was poured off since the organisms were settling to the bottom and the remaining water and sediment from several jars poured together. Examination of many small pools on the shore of this lake showed that Volvox was not present in many of them. Some pools yielded Pleodorina. No colonies were found in the water of the lake itself.

Smith (1907) who has collected Volvox in the vicinity of Ann Arbor has found it in "small glacial pools containing Riccia and duckweed" but apparently had not found it among sphagnum. He (Smith, 1905) found it best in permanent pools but apparently never in great abundance. He collected it by "dipping it up together with a little of the water" and also "by sweeping a bolting cloth net over water plants, or better, using a 'Birge net'". Smith's method of dipping up the water together with plant material has been found by me to be the best method for collecting the organism here in Michigan. While in Nebraska the writer found *V. perglobator* Powers in such great abundance and in such clean open water that it was best collected with a small dipnet covered with bolting cloth or India linen. In this way large quantities of water could be passed through the net in a short time.

The collections made on October 27, 1915, were brought to the laboratory in the pint jars filled almost to the top with the water in which the organisms grew, together with about a half inch of the decaying vegetable material from the same source. Arrived at the laboratory these jars, still covered, were placed on the outside window ledge on the north side of the laboratory where no direct sunlight could strike them. They were kept here until November 29 when ice began to form on the surface of the cultures. At this time all jars except three belonging to the writer were brought inside the building. The writer's cultures remained outside for some time longer. While outside the window the number of colonies had increased greatly but when brought inside the numbers steadily diminished and soon disappeared. One student, Miss Rose Mayer,

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placed her cultures in an unheated room, not in direct sunlight, where they continued to thrive for some time. An abstract from her report on her cultures is here given:

1915.

Oct. 27. Volvox collected. (Other data as given above.)

Oct. 28. Jars containing Volvox were placed outside of the window on the north side of the building.

Nov. 3. Volvox was multiplying.

Nov. 15. Still increasing in number.

Nov. 29. Cold outdoors, some ice on surface of water. As many colonies as could be gathered were preserved. The remainder were placed in an unheated room but not in direct sunlight, i. e., the windows (south) were covered with gauze to diffuse the light.

Dec. 13. A considerable increase had taken place. Another lot was removed and fixed. Remainder was returned to the cold room.

Dec. 18. Colonies were quite numerous, but were beginning to fall to the bottom of the jar.

Jan. 25. No colonies were observed. Since the last examination the temperature of the room had increased considerably.

When the cultures belonging to seven other students were brought inside they were so placed that they received some morning light. These cultures soon ran out and the writer has no further record of them. The writer's cultures (three pint jars) remained on the window ledge on the north side of the building until about December 5, but were protected at night with a cloth thrown over them. When brought inside they were placed on the window sill near the radiator. They received only north light. The colonies gradually disappeared but no record was kept of their last appearance. At various times the cultures were examined ocularly or with a hand lens but no colonies were seen until February 19 when a few colonies were seen in one jar only. They were noted again on February 26, and on March 7 the record states that they were in fair abundance. On March 16 the estimated number was 200 to 400 colonies and on March 27 several thousand were seen. At the time of writing, April 27, the water is green with them and they lie close together on the surface of the decaying vegetation.

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During the fall and winter no colonies in sexual stages were seen. On March 25 a single zygote was found in the debris from the bottom of the culture. April 25 several colonies with ripe zygotes were found but they were not numerous.

Of the other two cultures one was destroyed by accident about the middle of March but no colonies had appeared in it at that time. The other culture was observed from time to time and in it 3 colonies were found March 20, about 20 on March 25, and about 50 on March 27. April 20 there were several thousand colonies present. These cultures are being kept in the hopes that further information may be gained in regard to the culture of this organism.

In conclusion it should be stated that since these cultures were not kept under controlled conditions it is possible that this success could not be repeated. However, certain points in the culture of this organism may be emphasized, viz., the water for the cultures should be from the same source as the organisms. Never use tap-water for making up the culture or for making good evaporation. Keep the cultures covered to prevent evaporation and consequent change in density of the medium and to exclude the dust and bacteria. The presence of organic material seems to be beneficial. Direct sunlight is unnecessary and is to be avoided because it causes too great variations in temperature in closed vessels. North light is good; in fact many algæ thrive in it as evidenced by the good growth of algæ in these cultures. Low temperature, above freezing, in early winter seemed to favor development. Old cultures should not be destroyed unless they have become hopelessly foul but they should be kept and the organisms given a chance to reappear.

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Zoölogical Laboratory, GEORGE R. LA RUE.
University of Michigan.

A NEW EMBEDDING STAGE

A new electrically heated embedding stage prepared according to designs prepared by laboratory men in this university has been recently put on the market by Eberbach & Co. of Ann Arbor. The essential parts of this embedding stage (see cut, Pl. XXI, Fig. 2) are a transite base 17¾ inches long by 4½ inches wide mounted on three levelling screws, a copper stage made in two parts, 4 by 13 inches and 4 by 4 inches respectively, and under one end of the longer copper stage an electric heating unit. The heating unit may be wound for any voltage and to yield any desired temperature. Those in use in the Zoölogical Laboratory are designed for 110 v. alternating or direct current and the current requirement is 0.5 ampere. This yields a temperature of about 74° C. No regulator or rheostat or other provision for controlling or varying the temperature is provided but since the coil is situated under one end of the stage lower temperatures may be secured by moving the object away from coil. A scale to indicate the gradations of temperature could be attached if desired. In practice the coil is attached to a convenient electric receptacle near the paraffine bath and that part of the stage over the coil is heated sufficiently to melt paraffine in a few minutes. The embedding tray may now be warmed over the hot stage, filled with melted paraffine and moved to a point on the stage where the paraffine is kept just melted. Objects to be embedded are now transferred to the embedding tray, oriented, and the label inserted at the end of the tray with the legend towards the margin of the tray. Now the tray is gently moved to the unheated end of the stage where the paraffine is permitted to congeal on the bottom sufficiently to hold the objects in place. Then the tray is trans-

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ferred to a dish of cold water or alcohol standing at the end of the embedding stage and into which it is immersed as soon as the paraffine is cooled sufficiently to prevent the breaking of the surface film by the water.

The use of this embedding stage makes unnecessary the use of the top of the paraffine bath for this purpose. Its use helps greatly in securing good embedding because it permits the paraffine to be melted clear to the bottom of the embedding tray and thus the orientation is made easy. The plan of allowing the object to lie on a layer of congealed paraffine is not only unnecessary but is faulty in that the paraffine is too soft to permit accurate orientation of the object and also because a cleavage plane is formed at which the paraffine frequently breaks during the sectioning. The stage is convenient to use, does away with the necessity of using gas, and largely obviates the danger of overheating the tissue which danger is always present when a gas flame is used for heating the ordinary stage. This stage because of its low construction is very stable, unlike the very insecure stage used with the gas flame, and with the levelling screws it may be levelled. In several months' use by a class no objectionable features have appeared and its good points are only the better appreciated.

Zoölogical Laboratory, GEORGE R. LA RUE.
University of Michigan.

MAKING GLASS PLATES FOR COVERING MUSEUM JARS

At this time when it is impossible to secure from abroad the glass plates for covering museum jars it is worth while to know that after a little practise passable plates may be made in any laboratory equipped with power grinding and buffing machinery. Double strength glass plates may be purchased cut to size or they may be cut in the laboratory. Their edges may be rounded and a narrow ground surface at the margin may be secured by grinding on a carborundum wheel designated 120J-G5 which can be purchased from the Carborundum Co., Niagara Falls. The size of the wheel will depend somewhat on the power and speed of the grinder. In this laboratory a 4½ by ½ inch wheel belted to a ½ h. p. motor

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of about 1800 R. P. M. is used. While plates made in this way are not as good as the imported article they are usable and cheap, and by this means museum jars whose covers have been broken may be put into use.

Zoölogical Laboratory, GEORGE R. LA RUE.
University of Michigan.

THE POSSIBLE NATURE OF THE "BOOK LUNGS" OF SPIDERS

The abdomen of spiders is now unsegmented, and yet it is probable that spiders have descended from ancestors whose bodies were segmented throughout.

The breathing apparatus in spiders is varied, some forms showing some development of tracheal tubes. On the forward end of the abdomen are found two sacs, each of which encloses a folded membrane which exposes the blood to the air. These are the book lungs.

In the section of such a lung from an *Aglena* (Plate XX, Fig. 1) the membranous character of the organ will be seen. Red blood cells may be seen between the double membranes. The outer surface of the membrane is covered with short spines, which prevent the moist membranes from adhering.

It is possible that this arrangement is derived from an ancestral form which had external gills at this point, somewhat similar to the tracheal gill membranes of insect nymphs.

A figure of a section of the young wing membranes of an Ephemera nymph is shown (Pl. XX, Fig. 2) for comparison. The similarity of structure is striking.

E. W. ROBERTS.

NOTE ON THE NATURE OF THE CYTO-PLASTID

The cyto-plasm of a cell contains unit plastids which themselves bear a great resemblance to a complete cell with its nucleus and cyto-plasm.

Using the Tussock Moth egg for an illustration we get a suggestion of this condition. The egg is filled with nutritive material supplied by numerous nurse cells from their own cytosome system.

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FIG. 1. Book-lung of Spider.

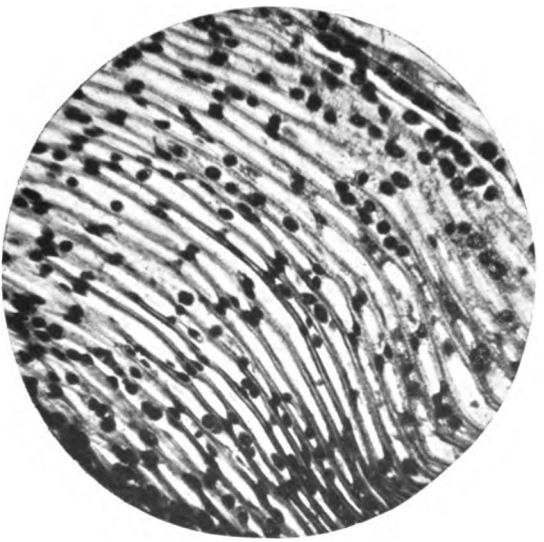

FIG. 2. Section of growing wing of Ephemerid Nymph.

PLATE XX

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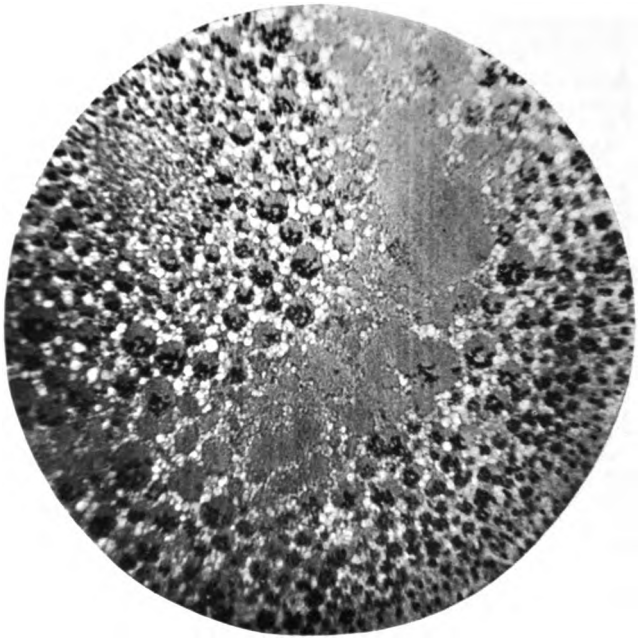

FIG. 1. Cyto plastid in Tussock Moth Eggs.

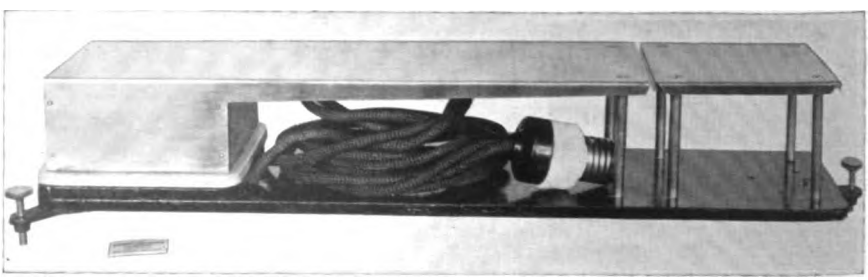

FIG. 2. Electrically heated embedding stage, Eberbach & Co.

PLATE XXI

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Into this mass of raw food material, are extruded from the walls of the oviduct certain granules which stain as nucleo-somes. These appear singly at first as seen at the edges of the photograph (Plate XXI, Fig. 1). These chromatic bodies seem to multiply by binary divisions and thus produce a progeny of various numbers of staining bodies.

These bodies sometimes group themselves into paired threads, and in some cases separate into distinct groups resembling the anaphase of nuclear mitosis. The cyto-plastids thus seem to act as a miniature cell, containing grouped staining bodies, a surrounding body of plasm, and a definite membrane.

These efforts of the staining bodies to divide and to group themselves gives one the impression that the process is a modified or incomplete sequence of what we see in mitotic divisions of the chromosomes, from which they may be derived.

In the lower animals such as Rhizopods and others, the nucleus buds out into the cytoplasm similar bodies, which undergo analogous binary divisions and finally form a large progeny of staining bodies. Later these group and form new nuclei which surround themselves with a plasmic body, about which a new cell wall is formed. In the Tussock egg these processes seem to stop short of this final result, and produce objects whose fate is to be used as food.

E. W. ROBERTS.

SENESCENCE AND REJUVENESCENCE

The fact that an organism at its individual beginning is "young" and gradually loses some of the powers of youth with the passage of time, is so commonplace that its real significance escapes us. The further fact that one of these older organisms can, in spite of its age, produce offspring which apparently do not have the age of the parent, but are as young as the parent originally was at the beginning, brings us acutely upon the problems at the basis of this book. In what does growing old consist? How does age differ from youth? When reproduction takes place, is youth absolutely restored? If so, do the old materials become young again, or is there some material within the aging body which does not itself grow old?

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Many investigators believe that youth is the eternal possession of the germ-plasm, and from this the cells of the body form,—these latter alone becoming aged. There is in this view only one process,—senescence. Rejuvenescence is apparent only.

Professor Child, thru the study of the lower forms particularly, in which differentiation is not so pronounced and non-sexual reproduction and regeneration are more frequent, comes to the conclusion that rejuvenescence is not confined to the debatable case of the germ cells, but is found as characteristic a feature of life as is senescence. He finds it also in agamic reproduction, regeneration of lost parts, in the restoration of starved tissues to activity; and the like. The author believes that senescence is not continuous therefore; that it is interrupted now and again by processes essentially restorative of youth; that there is no eternally youthful stuff, but that rejuvenescence and dedifferentiation are just as really a part of the life-cycle as is growing old.

As a criterion of youth one may take as his standard the normal changes which are observed in the embryo formed by the union of gametes,—e. g. rapid growth, cell division, differentiation and other vital processes. Senescence would imply on the other hand a decrease in these dynamic processes. Professor Child undertakes to erect a more exact standard based upon the principle that youth and age may be measured by the rate of metabolism in the organism. As a test of the rate of metabolism, and hence of the youthful or senile tendencies of tissues, the author holds that their susceptibility to certain chemical substances is satisfactory. In solutions strong enough to kill in a short time, the more actively metabolic (younger) objects are killed first; whereas in weaker solution to which acclimation is possible, those more metabolic live longer.

The captions of the five principal divisions or parts will sufficiently indicate the order and scope of the work, which is in large part based upon the author's original researches here given to the world for the first time:—I. The Problem of Organic Constitution; II. An Experimental Study of Physiological Senescence and Rejuvenescence in the Lower Animals; III. Individuation and Reproduction in Relation to the Age Cycle; IV. Gametic Reproduc-

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tion in Relation to the Age Cycle; V. Theoretical and Critical. There are seventeen chapters in all.

The book is a worthy illustration of a monographic treatment of a subject instead of in numerous detached papers. The bioiogical workers will be grateful.

Senescence and Rejuvenescence, by Charles Manning Child. Illustrated, 480 pages. University of Chicago Press.

A TEXT BOOK OF HISTOLOGY

Messers Jordan and Ferguson, in this book, have tried to give to students and teachers a treatment of the relatively stable matter of histology which will overcome, thru interest, the difficulties which the average student has in approaching and mastering the subject. There is no doubt that histology may be, and often is, so presented as to be deadening and full of drudgery. Indeed in the opinion of the reviewer this is an indictment that will stand against very much of the work done in College and University laboratories in America today, not alone in histology but in all aspects of morphological work. The writers justly conceive that such interest may be stimulated and held by relating the facts of structure to their practical ends in terms of function; and to their meaning in terms of the generalizations which give vitality and zest to investigation. The student of any science is entitled to the pleasure that comes naturally from following his discoveries on thru into conclusions that relate his facts into a system. Whenever it has been necessary, in order to accomplish this, the authors have not hesitated to introduce the facts of development and of function, and the theoretical explanation which will enable the student to appreciate the facts. This is illustrated, for example, by the discussion of the neuron theory under nervous tissues, and the theories of the inversion of the vertebrate retina in the discussion of the eye. In the opinion of the reviewer they have had good success in accomplishing their announced purpose.

The order of discussion will be made clear by giving the chapter headings. An introductory chapter deals with protoplasm and the cell. Then follow chapters on Epithelial Tissues; Connective Tissues; Muscular Tissue; Nervous Tissues; End Organs; Blood Vessels; Blood; Lymphatic System; Mucous Membranes and

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Glands; Skin; Respiratory System; Digestive System; Urinary System; Reproductive System; Ductless Glands; Nervous System; Eye; Ear. A chapter of 40 pages on Histological Technic concludes the book.

The illustrations are well chosen, excellently executed and liberal. The text is well written and clear. Mechanically the book is a beautiful one.

A Text Book of Histology, by H. E. Jordan and J. S. Ferguson. 800 pages, 594 illustrations. D. Appleton & Co., New York.

MEDICAL AND VETERINARY ENTOMOLOGY

It has long been recognized that the richest ore is often found where two veins intersect. Under the title Medical and Veterinary Entomology, Professor Herms treats the territory common to Economic Entomology and Parasitology. This treatment, in the hand of one who has himself made noteworthy contributions to both fields, in both a scientific and a practical way, assures a book helpful to those immediately interested in the subjects and illuminating to the general zoologist.

The author expresses his purpose as being to systematize the subject and thus assist in securing for Medical Entomology a place among the applied biological sciences, rather than to supply a comprehensive treatise. He indicates that he hopes to be of special service to the physician, the veterinarian, the health officer and sanitarian, as well as to the teacher and student.

Biologically the field includes the morphology and ecology of insects (and of the ticks and mites which are embraced in the treatment); their relations to the host when they are the direct causes of the diseases; the biology of the bacteria and protozoa by which the diseases are caused when the insects are merely the carriers of the germs; the physiology and pathology of the animals attacked. While in itself a specialization, it thus rapidly broadens out into the general interests of biologists, theoretical and practical, when its connotations are realized.

The content and mode of treatment are apparent from an examination of the chapter headings:—Introduction; Parasites and parasitism; Insect anatomy and classification; Insect mouth parts;

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How insects carry and cause disease; Cockroaches, beetles, thrips; Lice; Bedbugs; Mosquitoes as disease bearers, and their control; Buffalo gnats and horseflies; Common house fly; House fly control; Bloodsucking Muscids; Myiasis; Fleas; Ticks; Mites. An interesting final chapter is given to the discussion of venomous insects and arachnids,—as bees, wasps, spiders, scorpions, etc. The nature of the venom, the manner of its introduction, and its effects are treated.

The book is well illustrated with half-tones, has numerous keys for the identification of the principal genera, and gives the best accepted treatments for control of the insects and of the resultant diseases.

Medical and Veterinary Entomology, by William B. Herms. 394 pages, illustrated. The Macmillan Co., New York, 1915. Price, $4.00.

CLASSIFICATION OF LEPIDOPTEROUS LARVÆ

Number one of Volume two of the Illinois Biological Monographs bears the above title, and is the thesis of the author, S. B. Fracker, offered in the Graduate School of the University of Illinois toward the degree of Doctor of Philosophy in Entomology.

The paper is divided into two parts; the first being devoted to the question of the homology of the setæ of the larvæ of Lepidoptera, and the second to the systematic outline of the families and genera.

The position taken by the author, that a final classification of the insects based upon both the larval and adult characters necessarily eliminates errors that belong to a classification based solely on either, must commend itself to the general student. It is surely true also that anything which will make identification of insects more possible in the larval stages will save much trouble and time required to rear them to maturity for more certain identification.

The proffered classification is based largely upon the setæ and the armature developed in connection with them, the head parts, the size and shape of the spiracles, the prolegs and the hooks they bear, and other structures somewhat less certain.

The portion of the paper that will prove most suggestive to the general biologist is the discussion of the setæ, the method of homologizing them, and the evidence for their sufficient identity for comparative purposes.

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The first step is to get a standard or type segment so far as setal arrangement is concerned. This is done by taking the various thoracic and abdominal segments in the more generalized members of the two sub-orders, and plotting from these the setal arrangement by superimposing them so as to get a composite. This composite gave about 15 setæ with approximately the distribution found on the pro-thorax of the most generalized and primitive types. With this composite all the different segments of every larva were compared. In a similar way a type of abdominal segment is worked out and used as a standard.

The author concludes that the first-stage larva, before entering upon the various moults, best represents the ancestral type of Lepidoptera and that the setal arrangement of the first instar is essentially the same as in the ancestors, and thus serves as a connecting link between the more generalized type and the modern, specialized older stages.

The various tests of homology of setæ used by the author are:—(1) similar grouping of setæ in mature caterpillars generally; (2) similar position of setæ on certain segments of modern mature caterpillars; (3) similar arrangement of setæ on all the segments of generalized groups of caterpillars; (4) similar arrangements on all the segments of newly hatched larvæ; and (5) evidences of migration from these similar positions.

The author believes that the setal arrangement of every segment of the body of the larvæ of Lepidoptera has been derived from the same ancestral type; that 12 such primary setæ can be homologized; that these primary setæ are present in the first instar; that they may be modified by loss chiefly (abdominal segments) or by loss and change of position. Sub-primary setæ appear in later instars and may become associated with the primary setæ in ways more or less confusing. They may develop in tuffs of various kinds.

The systematic part is based upon maturer larvæ, but the characters apply for the most part to the earlier also. Elaborate keys of the order and of the families, leading to the genera, are supplied.

Classification of Lepidopterous Larvæ, Stanley Black Fracker. Illinois Biological Monographs, Vol. II, No. I; July 1915. University of Illinois. 170 pages, 10 plates. Price $1.50.

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JULY
1916

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Beloit, Wisconsin.

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NOTICE TO MEMBERS:

Business Sessions of the *American Microscopical Society* will be held at New York City in connection with the American Association for the Advancement of Science. See Program of A. A. A. S. for time and place.

T. W. GALLOWAY, Secretary.

Beloit, Wisconsin.

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THE INNERVATION OF THE AMPULLÆ OF LORENZINI IN ACANTHIAS VULGARIS

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Introduction

In a former paper (1914) the author gave a general description of the ampullæ of Lorenzini in Acanthias vulgaris, presenting evidence as to the sensory nature of these organs. Since that time further work has been done on their innervation and is embodied in this paper.

Peabody (1897) published a short article on the innervation of these structures as shown by methylin blue preparations, and the present author's findings as published in the 1914 paper closely agreed with his in the main, altho there was some doubt expressed at that time as to the nodules described being the actual termination of the nerve fiber. Later findings indicate that a complete impregnation was probably not present either in Peabody's preparations, or in the author's in 1914. Later methods have demonstrated the final terminations of the nerves supplying the ampullæ.

Methods

New material impregnated by the methylin blue method was sectioned, and several preparations were made by the Bielschowsky double silver and gold process. The results therefore, were checked

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up between those two methods, as well as by maceration and isolation of cells.

It was considered probable that if the nerves which supply the ampullæ terminate on particular cells in the ampullary pockets, isolation by means of maceration and teasing would be likely to show isolated sensory cells with their nerve terminations attached. Material was macerated in 3% caustic potash for 24 hours, teased immediately in glycerine and mounted in glycerine jelly; or carried through the alcohols, teased in balsam and mounted. In this way thousands of isolated sensory cells were obtained as well as isolated supporting cells. This proved to be a very valuable method of studying the general structure of the epithelium as well as for the sensory cells with their nerve terminations.

The Gross Innervation of An Ampulla

There are eight definite groups of ampullæ in Acanthias; two on the dorsal surface, four on the ventral, and two on the lateral surfaces of the snout and head. As might be expected these groups are bilaterally symmetrical. The number in each group, however, varies somewhat and each ampulla has not a fixed position on each side. The position of four of these groups may be seen in Figure 2 (dorsal Fig. 2-A, ventral Fig. 2-C.) The two ventral groups have not yet been divided into four by the cartilege bars of the rostrum.

There are two kinds of ampullæ in Acanthias. Those with a single duct extending from the alveolar portion to the surface, and those with a split duct. As was explained in the previous paper these two types are essentially alike, only in the ampullæ which have a double duct there are no primary ducts, the division between the two secondary tubules being carried completely to the surface.

The model in Figure 1 (Pl. XXII) was made from sections 5 microns thick by the wax plate method and shows approximately the size and shape of the ampullæ having a double duct. In this case an ampulla having a short duct leading to the surface was selected, in order that the entire course might be modelled. This particular ampulla had alveoli and does not follow an equal division

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into secondary and tertiary ducts. There are several alveoli which come off the main or secondary ducts. (Fig. 1-D.)

The external opening is nearly round and divided by the partition between the two secondary ducts. Immediately below the skin the duct widens out to about twice the size of the opening, and proceeds downwards to the alveolar portion. Here there is a sharp constriction to about seven eighths its size and then the alveoli are budded off at different levels. This fact is important for it can be readily seen that the number of alveoli cannot be counted from a single section, as they will not all be included. The average number of alveoli in the ampullæ of Acanthias is twenty-two. The model shows the general appearance and proportion faithfully with the exception that there is some slight shrinkage so that the alveoli in the fresh total mount are somewhat more rounded and not so long in proportion to their width. This is probably due to the coagulation of the mucus in the lumen of the ampulla due to the fixing fluid.

The ampullæ in the two groups on the dorsal surface of the snout are innervated by the ophthalmicus superficialis; those of the ventral groups by the buccalis; and those of the spiracular region by the mandibularis externus branches of the seventh or facial nerve. These branches also innervate the lateral line system in those regions. The ophthalmicus superficialis may be seen under the dorsal group in Figure 2-B, and the buccalis directly over the ventral group in Figure 2-D.

From the main nerve trunk as it passes along close to the alveolar portions of the ampullæ, there is given off a single twig to each ampulla containing from 5 to 15 medullated fibers. The number of medullated fibers does not correspond at all to the number of alveoli, altho in cases where there are a large number of alveoli there is a large number of fibers. Roughly speaking there are about twice as many alveoli as there are fibers supplying the ampulla. These twigs run to the ampulla, and as the alveoli are arranged in a circle, there is a central space into which the nerve twig runs. (Figs. 5 and 6.) This twig does not give off branches until it reaches a level just below where the duct begins and where the ampullary pockets are budded off. At this point the nerves bend laterally (Fig. 5-b) with respect to the ampulla,

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and radiate out between the alveoli close to where the alveoli join the duct. (Fig. 6.)

This radiation takes place practically at the same plane for all of the nerve fibers, and all turn at right angles to their first course in 15 to 20 microns.

While doing this, the nerve fibers, which have up to this time been medullated, lose their sheaths, and when they appear on the external surface of the ampulla, between the alveoli, they are entirely devoid of covering. (Fig. 3.) Thus it may be seen that the course of the neurofibrils over the ampullary pockets themselves is in the majority of cases, from the bases of the alveoli toward their distal ends, speaking in respect to the orientation as shown in the model. (Fig. 1.)

As the neurofibril reaches the external surface of the ampulla, it divides into a great number of neurofibrillæ which anastomose over the entire surface of the pockets making a network over that portion of the ampulla which is sensory in character. (Fig. 4.) Whenever two fibrillæ branch a spot or nodule is formed. Nodules are also often seen along the course of the fibrils, as well as where a fibril apparently ends. (These nodules are labelled *b* in Figures 9 to 14.) These were taken by Peabody (97) and by the author (1914) to be the endings of the neurofibrils upon the bases of the sensory cells. This, however, I have now seen is not the case.

Nerve Terminations

The sensory cells in the epithelium are well seen both in sections and in isolated specimens, and are the large pear-shaped cells with broad bases and a short projection which extends out to the lumen of the ampulla. Each of these cells is surrounded by four or five supporting cells which fit over the top as shown in Figure 9. In macerated preparation the pear-shaped cell comes away from the supporting cells and leaves the small hole where its projection reached the lumen. (Figs. 7-a and 8-a.) The supporting cells in macerated preparations give a table-like appearance as shown in Figure 8, and they occasionally stick together as in Figure 7. Figure 9 is a drawing of a sensory cell complete with its four supporting cells and its nerve termination. All of these cells are more

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or less rounded due to the fact that they swell somewhat in the maceration process. This gives a more or less rounded appearance to their bases which appear flat in sections. Figure 10 is a drawing of an isolated cell which was cleared and drawn in optical section so as to show the small projection from its upper surface to the lumen.

The neurofibril, which has been traced to the small nodule on the base of the cell, does not stop there but has a small process extending up between the sensory cell and the interstitial cell. (Labelled *c* Figs. 9-14.) This process does not readily impregnate with methylin blue but can be seen occasionally by very careful focussing with the oil immersion lens in cases where it does occur. It has been seen a sufficient number of times to justify the statement that in all probability all of the pear-shaped cells have such a termination. This process which was entirely overlooked in the previous paper, is the final termination of the nerve. This process arises from the nodule on the base of the cell and is very slender. It extends to the level of the lower border of the nucleus and there flares out with a fork and encloses an area which in my preparations appeared lighter than the rest of the nerve. It must be understood that this oval area is NOT on the inside of the cell, but closely applied to its external surface. This form of ending is much like the terminations of the sensory fibers in the ear of Mustelus as described by Morrill (97), and may be taken as evidence of the homology of these ampullæ with those of the ear.

In isolated sensory cells impregnated by the methylin blue method the cell may sometimes be seen with this process connected with the basal nodule as well as with part of the torn neurofibril. (Figs. 11 and 14.) Morrill (97) in his figures shows many cells which have no hairs, and yet were sensory in character. These cells in the ampullary pockets look somewhat like hair cells with the exception that the process is rather thick and does not project out into the lumen. In every case, however, it connected with the lumen. This is not as readily seen in sections as it is in isolated specimens, because in sections these sensory cells are so large that it is only occasionally that a section cuts the process exactly to show it extending to the lumen. In thick sections, by focussing, it may

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be recognized. It may thus be seen that the real termination of the nerve supplying the ampullæ of Lorenzini in Acanthias is a slender process extending from the basal nodule to a point about half way up on the cell, and there flaring out into an oval plate in close contact with the sensory cell.

Summary

1. Each ampulla is innervated by 5 to 15 medullated fibers from the seventh or facial nerve.

2. These fibers run into the central space of the ampulla, turn sharply and radiate laterally to reach the external surface of the ampulla.

3. From there they radiate downwards forming a network with nodules on the bases of the sensory cells.

4. The termination in Acanthias vulgaris is a light oval plate closely applied to the sensory cell at about its middle and connected with the basal nodule by a slender strand.

I wish to thank Dr. J. B. Johnston for his kind help during the course of this investigation.

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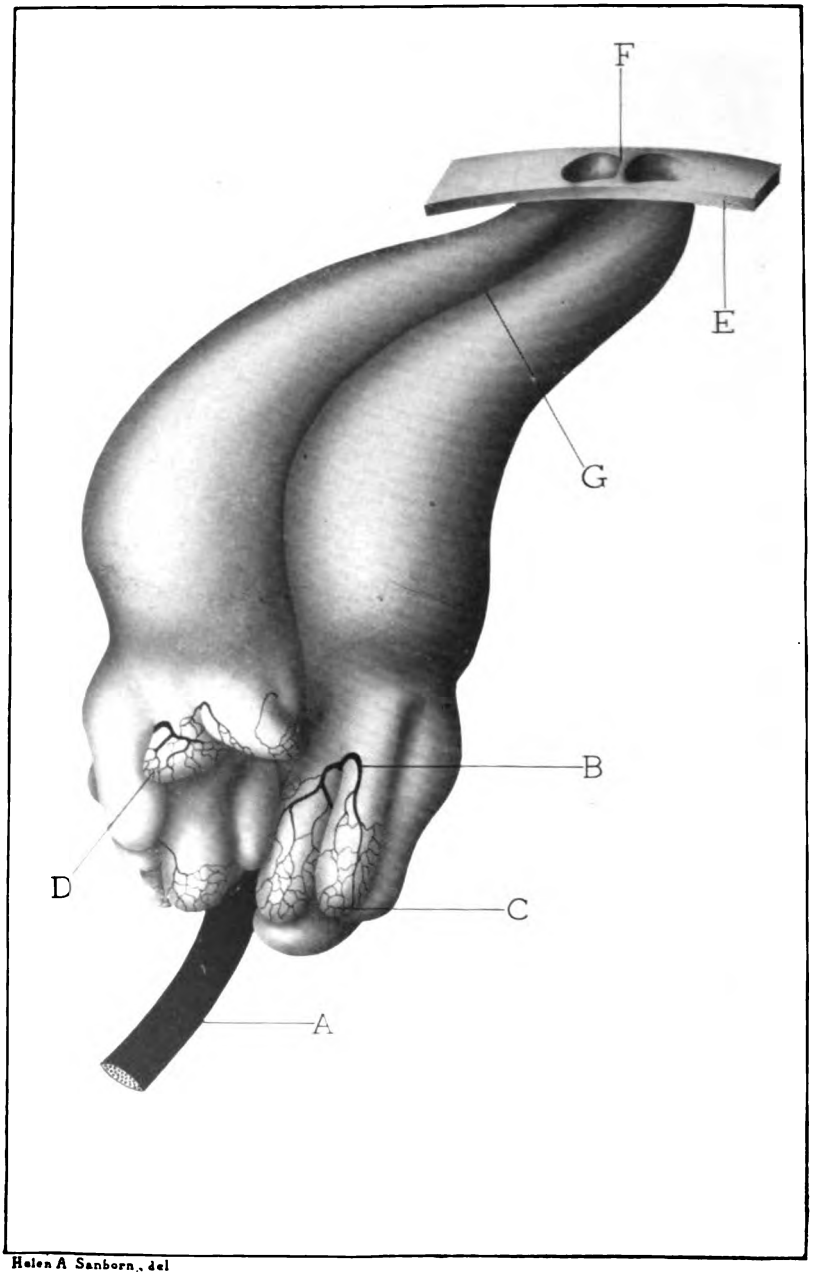

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PLATE XXII

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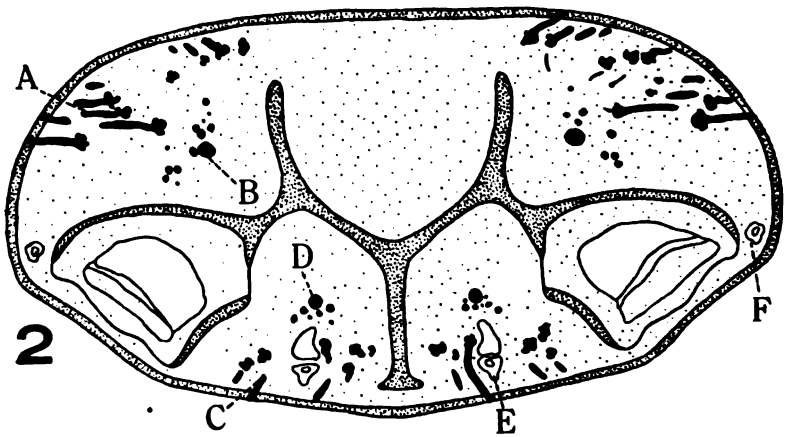

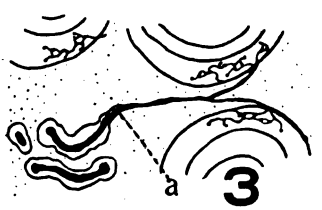

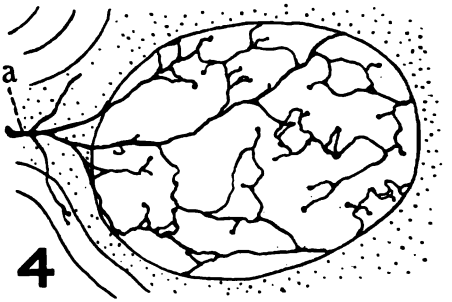

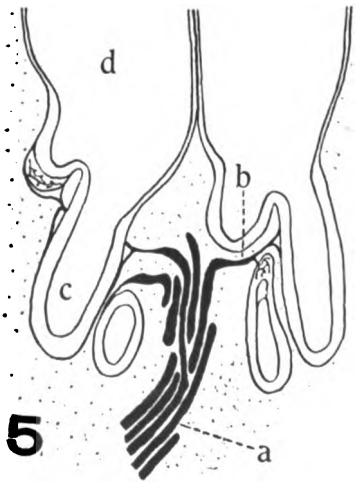

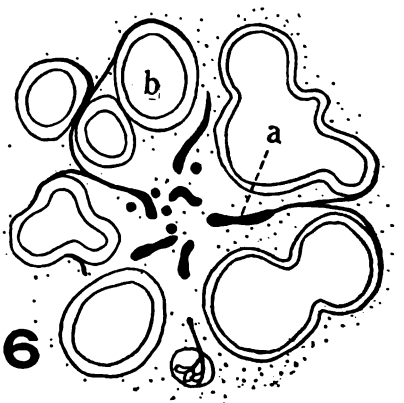

PLATE XXIII

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DESCRIPTION OF PLATES

PLATE XXII

Fig. 1. Drawing of a wax model of an ampulla having a double duct. A—nerve bundle entering central space. B—ramification of unmedullated fiber over alveolus. C—anastimosis of neurofibrils over distal end of alveolus. D—alveolus coming directly off secondary duct. E—skin of fish. F—partition between the two secondary ducts and openings.

PLATE XXIII

Fig. 2. A transverse section of an Acanthias pup just forward of the nasal capsules. A—Dorsal group of ampullæ. B—Ophthalmicus supersuperficialis. C—Ventral group of ampullæ. D—Buccalis nerve. E—Lateral line system. F—Lateral line system. X 15

Fig. 3. Drawing showing the loss of the medullary sheath as the nerve bends laterally. Bielschowski method. a—point where loss of sheath occurs. X 180

Fig. 4. Showing the anastimosis of the neurofibrils over the ampullary pocket. Methylin blue impregnation. a—point where nerve leaves sheath. X 250

Fig. 5. Diagram to show nerve entrance and lateral radiation. Longitudinal section, Bielschowski method. a—nerve twig. b—nerve radiating laterally. c—ampullary pocket. d—duct. X 180

Fig. 6. Transverse section showing nerves radiating laterally. Bielschowski method. a — nerve radiating laterally. b — ampullary pocket. X 180.

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Plate XXIV

Fig. 7. Group of supporting cells from which three sensory cells have been removed by maceration. a—opening by which the sensory cell connected with the lumen.

Fig. 8. Group of supporting cells from which one sensory cell has been removed by maceration. a—opening by which the sensory cell connected with the lumen.

Fig. 9. A group of supporting cells enclosing a sensory cell with its nerve termination. a—projection of the sensory cell to the lumen. b—basal nodule. c—nerve termination.

Fig. 10. Optical section of an isolated sensory cell with three supporting cells, and nerve termination. a—process extending to lumen. b—basal nodule. c—nerve termination.

Fig. 11. A sensory cell isolated by maceration to show process and nerve termination. a—process extending to lumen. b—basal nodules. c—nerve termination.

Fig. 12. Section directly through the process extending to the lumen showing nerve termination on side of cell, and two supporting cells. Methylin blue impregnation. a—process. b—basal nodule. c—nerve termination.

Fig. 13. Section directly through the process extending to the lumen showing nerve termination on middle of cell, and two supporting cells. Lettering as in Figure 12.

Fig. 14. A sensory cell isolated by maceration to show process and nerve termination. Lettering as in Fig. 11.

All figures in Plate XXIV were drawn with the oil immersion lens and have a magnification in the plate of 2000 diameters.

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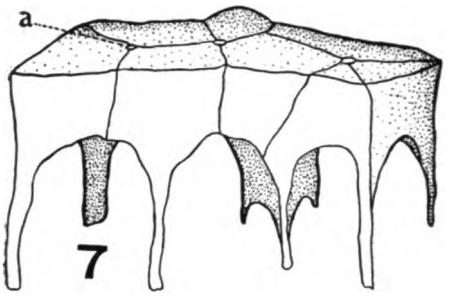

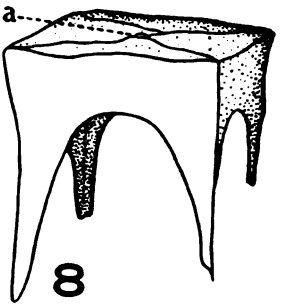

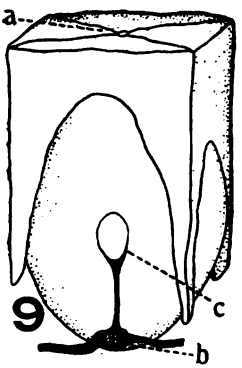

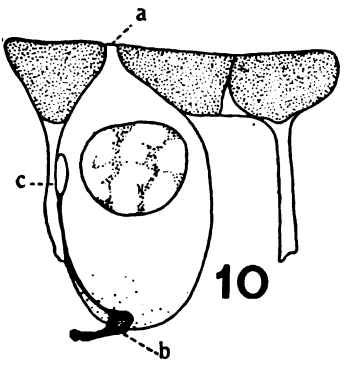

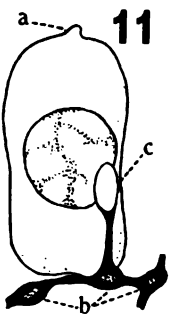

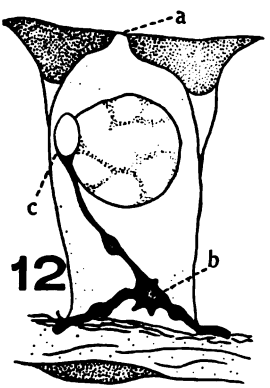

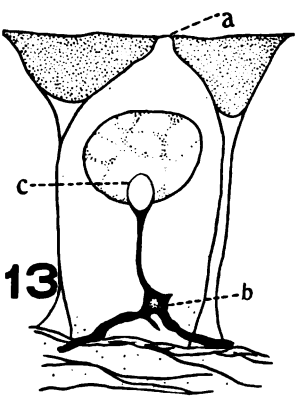

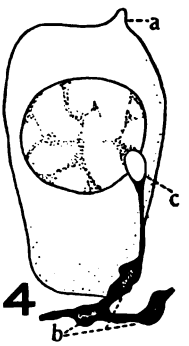

PLATE XXIV

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A NEW MONOSTOME TREMATODE PARASITIC IN THE MUSKRAT WITH A KEY TO THE PARASITES OF THE AMERICAN MUSKRAT*

By FRANKLIN D. BARKER
Professor of Parasitology, the University of Nebraska

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INTRODUCTION

In studying the parasites of muskrats shot on Lake Chisago, Minnesota, last summer (August, 1915), in addition to several species previously reported for muskrats Barker (:15, p. 184) more than a hundred specimens of a small monostome trematode heretofore unrecorded for the muskrat were found in one male animal.

MORPHOLOGY

To the unaided eye the trematodes appear as very small, thick ovals or globules, milk white in color. The length varies from 0.709 to 0.899 mm., the breadth from 0.501 to 0.657 mm. Under the hand lens or binocular the anterior third or fourth of the body is seen to taper gradually. The posterior third of the body also tapers slightly, but the end is markedly truncate. A rather wide but shallow indentation is fairly constant on each side in the region of the middle third of the body, and the body is widest just anterior to this constriction. A slight, wide but shallow, indentation also occurs in the center of the posterior end of the body, which marks the outlet of the excretory vesicle. The dorsal surface of the body is strongly convex in both diameters; the ventral surface is concave or cupped.

The lateral and posterior margins of the body turn ventrad and mesad forming a ventral horse-shoe-shaped shelf one-fifth to one-

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fourth the width of the body. The anterior fifth of the body is often strongly flexed ventrad, this flexed condition together with the ventral shelf forms a characteristic elongated ventral cavity or cup-like groove which undoubtedly functions as an effective holdfast in lieu of an acetabulum, which is absent in this form. The ventral groove is wide and shallow anteriorly and narrow and deep posteriorly. In some specimens the anterior end of the body is straightened out obliterating the cavity at that end. The posterior end likewise may be straightened, thereby breaking the continuity of the shelf and altering the shape of the ventral depression. Ventral papillæ or ridges are entirely absent and the body is devoid of spines or spinelets.

Digestive tract: Only one sucker, oral in position is present. It is slightly sub-terminal, quite muscular, circular or oval in shape and measures 0.05 to 0.06 mm. long by 0.052 to 0.065 mm. wide. The esophagus is straight and about twice as long as the diameter of the oral sucker. A pharynx is absent. The intestinal caeca lie in the median third of the body, and are slightly undulating but without pockets. Their course is nearly straight to the posterior half of the body where they make a decided wide outward bend, but in the posterior fifth of the body they again turn medianward and approach each other but remain separated by a median space equal to about four times their diameter. Both caeca end blindly in the posterior eighth of the body.

Genitals, female: The ovary lies in the extreme posterior end of the body, just to the left of the median line and dorsal to the left testis. Its bulk lies between the posterior ends of the testes, but a small portion overlaps one testis. The ovary is elongated and somewhat convoluted or twisted and the convolutions do not all lie in the same plane which gives a lobed or segmented appearance to the organ in any one plane of focus. The oviduct arises from the internal lobe of the ovary and almost immediately penetrates the shell gland and emerging as the uterus proceeds with several coils anteriorly and to the left around the base of the cirrus pouch. It then passes transversely across the entire body of the worm and in increasingly larger ascending transverse coils extends anteriorly to the level of the bifurcation of the digestive tract, then descend-

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ing in wide transverse folds or coils to the level of the cirrus pouch again it passes posteriorly in a sharp right angle turn around the base of the pouch and merges with the vagina. The vagina is narrower than the uterus and lies just posterior to and parallel with the cirrus pouch. The lumen of the terminal third of the vagina is widened and its wall wrinkled affording considerable expansion for the reception of the unusually large thick cirrus. The vagina terminates externally in a large opening the vulva, or female genital pore, slightly posterior and to the left of the opening of the cirrus pouch. The lumen of the terminal portion of the vagina is lined with thick cuticula and surrounded by two muscular sheets, one of longitudinal and one of circular muscle fibers. This muscular wall is in turn surrounded by a large oval mass of gland cells. In many specimens the lumen of the vagina and the contiguous transverse descending arm of the uterus are filled with sperm cells. The vitellarium consists of two compact convuluted tubular masses extracæcal in position, one lying on each side of the body in the middle third. From the posterior portion of each gland a transverse vitelline duct passes posteriorily and mesad and enters a small but definite oval vitelline reservoir median in position and lying between the fourth and last posterior fifths of the body just anterior to the shell gland. Neither a Laurer's canal nor a receptaculum seminis were found.

The shell gland lies in the median plane of the posterior fifth of the body between the testes. It is triangular in shape, fairly large, and well defined, tho not prominent. The oviduct passes thru the center of its mass, the cells of which are arranged in a characteristically radiating manner around the lumen of the oviduct.

Eggs: The eggs are very numerous, of a light straw color, oval in shape and about twice as long as wide. The length varies from 0.02 to 0.024 mm., and the breadth from 0.010 to 0.013 mm. A well defined operculum or lid is present at the narrower end of the egg. At each pole the shell substance is drawn out into a very long solid tapering filament. Each polar filament is often five times as long as the egg proper. The filaments of the eggs *in utero* intertwine forming tenacious strings of eggs which can withstand considerable tension. The manner in which these long polar filaments

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are formed in the moulding of the egg shell offers an interesting problem.

Genitals; male: The two large testes are lateral in position, extracæcal, one lying on each side, in the posterior third or fourth of the body. They are solid oval or longated bodies longer than wide and are frequently two to five lobed. Their entire mass does not lie in the same vertical plane of the body. The left testis is generally larger than the right one. From each testis a short transverse vas efferens runs medianward, the two uniting to form a wide tubular convoluted seminal vesicle having its base in the median plane at the level of the anterior margin of the shell gland. From this point it passes forward turning to the right around the base of the cirrus pouch which it enters from the dorsal surface. The cirrus pouch is comparatively very large, its length being one-third to one-half the width of the body and lies transversely across the median portion of the body at the anterior level of its posterior half. It is pear-shaped with its base to the right. The base contains an enlargement of the seminal vesicle which is surrounded by the cells of the prostate gland. The middle portion and neck contain the large thick cirrus. The lumen of the cirrus is markedly eccentric, lying nearer the under or ventral surface, and is lined with a thick cuticula. Internal to this lining is a single sheet of longitudinal fibers which aid in the retraction of the cirrus. The bulk of the cirrus is composed of parenchyma tissue and its musculature is poorly developed. No cirrus spinelets are present. The extruded cirrus is always markedly flexed or curved.

Excretory system: A slight depression occurs in the middle of the posterior end of the body and in the center of this depression is a small excretory pore. A very short canal lined with cuticula and surrounded by a row of radiating gland cells connects the pore with a small stellate shaped excretory vesicle or reservoir. From the reservoir four to eight fine radiating canals lead off in all directions and planes. The excretory tubules immediately branch and rebranch, soon becoming so attenuated as to make it impossible to follow their course.

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Systematic Discussion

Kossack (:11, p. 553) in a thoro revision of the monostome trematodes, groups the five heretofore recognized families in two families, the *Cyclocœlidæ* and the *Notocotylidæ*. He clearly points out that the *Notocotylidæ* differ essentially from the other families of monostomes with respect to the following striking characters: the absence of an acetabulum; the presence of ventral glands (drüsenpakete); the absence of a pharynx; the position of the extracæcal testes and the intercaecal ovary in the same plane in the extreme posterior end of the body; the elongated vagina and cirrus sac, which incloses a part of the much convoluted seminal vesicle; the strongly developed vitelline glands lying lateral to the intestinal cæca in the posterior half of the body; the absence of a receptaculum seminis; the uterine coils posterior to the cirrus pouch, and the presence of a polar filament at each end of the egg.

Lühe, (:09, p. 33) created a new genus *Paramonostomum* to contain *Monostomum alveatum Mehl. Paramonotomum alveatum* Lühe, conforms to the diagnostic characters of the *Notocotylidæ* with the exception of the presence of ventral glands, which both Lühe and Kossack report they were unable to find either in toto preparations or in sections.

We seriously question the validity of creating a new genus for this species on a single important differentiating character, namely the absence of ventral glands. The number of rows of these ventral glands varies from two in *Notocotylus diserialis* Ssinitzin to three in *Notocotylus triserialis* Diesing and five in *Notocotyle quinqueseriale* Barker and Laughlin and their complete absence in a species might naturally be expected. Their absence then, it seems to us is of specific rather than generic import.

Linton (:10, p. 69) has described a new species representing a new genus of monostomes which he found in three species of tropical fishes. As described by Linton the characters of this new species tally with the characters given for the *Notocotylidæ* with the following exceptions: ventral glands are absent; "the genital aperature is on the left margin approximately at the anterior third of the body;" "the cirrus pouch is relatively large," and a seminal vesicle is present lying "anterior and dorsal to the ovary."

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The monostome which we have described has a number of the diagnostic characters of the *Notocotylidæ* such as, the absence of a pharynx; the caudal and parallel position of the extracæcal testes and the ovary; the strongly developed and laterally placed vitelline glands and the eggs with long polar filaments. On the other hand it differs essentially from all of the described species of *Notocotyliadæ* in the absence of ventral glands, with the exception of *Paramonostomum alveatum;* the posterior position of the cirrus pouch, vagina and genital pores; the shape and large size of the cirrus and pouch; the position of the uterine coils anterior to the cirrus pouch, and the compact convoluted vitelline glands. Notwithstanding these differences it obviously more closely resembles the *Notocotylidæ* than the *Cyclocœlidæ*. The question immediately arises as to what characters and what combination or complex of characters must be present in a species to rightly place it in the family *Notocotylidæ*. As Kossack aptly remarks "Man ist bei der Schaffung des Trematoden—systems induktiv vorgegangen." It is often difficult to determine what morphological characters are of real phylogenetic import and therefore afford a reliable basis for a natural classification and what characters are due possibly to environment and therefore may be expected to vary and can not be relied on in determining the true systematic position of a given species. It seems to us that such a complex of characters as the absence of a pharynx; the posterior position of the testes and ovary in the same transverse plane with the testes extracæcal and the ovary situated between them and the presence of polar filaments on the eggs may be considered of real phylogenetic significance and for this reason we do not hesitate to place this new species in the family *Notocotylidæ*. Kossack (:11, p. 554), divides this family into the two sub-families *Notocotylinæ* and *Ogmogasterinæ* on the difference in the character or arrangement of the ventral glands. In the *Notocotylinæ* they are in rows (Reihen Drusenpakete), in the *Ogmogasterinæ* the glands are arranged along long ribs ("Längsrippen, auf denen Drüsenpakete ausmünden"). We agree with Kossack that such a division is well founded and further propose the creation of a third sub-family to contain such species as those described by Linton and the present paper and others that may be found later which have the general character complex of the *Notocotylidæ* but differ from the

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two recognized sub-families with respect to particular characters such as the position of the genital pore; the character of the cirrus; the nature and extent of the vitelline glands and the uterine coils and the absence of ventral glands. For this new sub-family we propose the name *Nudacotylinæ* (devoid of cups) and designate the following characters as diagnostic.

Sub-family Nudacotylinæ

Small *Notocotylidæ,* with thick bodies and without ventral glands. Ventral surface may be strongly cupped. Genital pore lateral (to right or left of median line) decidedly posterior to the intestinal bifurcation. Cirrus pouch large, thick, pear-shape, enclosing a small portion of a convoluted winding seminal vesicle. Vitelline glands strongly developed, compact convoluted masses, extracæcal and lateral in the posterior half of the body.

Uterus strongly developed, transverse folds extend laterally over the intestinal cæca and lie anterior or posterior to the cirrus pouch. Eggs with long polar filaments.

Type genus: *Nudacotyle,* mihi; other genus, *Barisomum* Linton. As the type species of the genus *Nudocotyle* we designate the form described in the present paper which we have named *Nudocotyle novicia.*

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KEY TO THE PARASITES OF THE AMERICAN MUSKRAT

Body flat, unsegmented III
Body flat, segmented II
Body cylindrical, unsegmented I

I. Body differentiated into long slender region and shorter thicker region 2
1. Body not differentiated into two regions. Body stiff, opaque; mouth surrounded by prominent lips..... b
a. Body thread-like; mouth not surrounded by prominent lips.
(1) Body hair-like (capillary): male with small bursa and single long spicule; eggs with polar plugs.....
.....*Capillaria ransomia* (Barker, 915: 197)
(2) Body thread-like; male with well developed bursa and two short spicules; eggs without plugs.....
.....*Trichostrongylus fiberius* (Barker, 1915: 196)
b. Body stiff, opaque; mouth surrounded by three prominent lips
.....*Ascaris* sp.[1]
2. Body stiff, opaque, divided into long slender cephalic region and shorter, thicker body region.....
.....*Trichuris opaca* (Barker, 1915: 195)

Species inquirendæ.

Filaria sp. (in collection of Bureau of Animal Industry, Washington, D. C.)

II. Found in cysts; in liver..... 2
Not in cysts; in intestine..... 1
1. (1) Body thin, flabby; genital pores unilateral; single row of hammer-shaped hooks on rostellum; three large testes present .....*Hymenolepis evaginata* (Barker, 1915: 194)
(2) Body thick, stiff; genital pores alternate; double row of long and short hooks on rostellum; testes numerous.....
..... *Anomotænia telescopica* (Barker 1915: 194)
2. Bladder-like cysts in liver.....
Cysticercus fasciolaris (Stiles and Hassall, 1894; Linton, 1915: 46)

III. Two suckers, oral and acetabular, present..... 2
One sucker, oral or caudal, present..... 1
1. Oral sucker not present..... B
Oral sucker present..... A
A. Ventral papillæ absent..... b
Ventral papillæ present..... a
a. (1) Three longitudinal rows of ventral papillæ present.....
.....*Catatropis filamentis* (Barker, 1915: 190)
(2) Five longitudinal rows of ventral papillæ present..*Notocotyle quinqueseriale* (Barker and Laughlin, 1911: 261)

[1]Unpublished research by Bessie Noyes.

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b. (1) Body thin, spatulate.....
.....*Monostomum affine*[2] (Leidy, 1858: 110-112)
(2) Body thick, ventral aspect cupped.....
.....*Nudacotyle novicia* (the present paper)
B. Caudal sucker present
(1) Testes branched. *Cladorchis (Stichorchis) subtriquetrus*= *Amphistomum subtriquetrum*[2] (Leidy, 1888:126-127)
(2) Testes slightly lobed.....
.....*Wardius zibethicus* (Barker, 1915: 192)
2. Body divided into cephalic and caudal regions..... B
Body not divided ..... A
A. Oral sucker surrounded by collar, bearing spines..... b
a. Oral sucker without collar.....
.....*Plagiorchis proximus* (Barker, 1915: 192)
b. Eggs numerous ..... bb
aa. Eggs few (30 to 100).....
.....*Echinoparyphium contiguum* (Barker, 1915: 187)
bb. Body 3 to 8 times longer than wide..... bbb
aaa. Body 10 to 15 times longer than wide.....
.....*Echinostomum coalitum* (Barker, 1915: 185)
bbb. Uterine coils definitely transverse..... bbbb
aaaa. Uterine coils compact.....
.....*Echinostomum callawayensis* (Barker, 1915: 188)
bbbb. (1) Anterior testis immediately behind the shell gland...
.....*Echinostomum armigerum* (Barker, 1915: 189)
(2) Anterior testis separated from shell gland.....
....*Echinostomum echinatum* (Leidy, 1888: 126-127)
B. Body divided into cephalic and caudal regions.....
.....*Hemistomum craterum* (Barker, 1915: 191)

[2]We question the correctness of the identification of this species and surmise that it is a species of Notocotyle.

[3]The identification of this species is questionable and we surmise that it is *Wardius zibethicus*. We wish to express our thanks to Doctor Joseph Leidy, Jr., for his efforts to secure for us the original material of Professor Leidy and regret that it has been misplaced or destroyed and is not available for comparative study.

SUMMARY

1. A new species of monostome trematode, *Nudacotyle novicia*, from the intestine of the American muskrat is described with four figures.
2. The suggestion is made that *Paramonostomum alveatum* Lühe rightly belongs in the genus *Notocotyle*.
3. A new sub-family, *Nudacotylinæ* is created under the *Notocotylidæ* with *Nudacotyle novicia* as the type genus and species.
4. A key to the parasites of the American muskrat is given.

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Explanation of Plate XXV

Fig. 1.—*Nudacotyle novicia* Barker, free hand drawing of unstained and uncompressed specimen, ventral aspect, under binocular. OS, oral sucker; C, cirrus, extruded; GP ♂, genital pore, male; GP ♀, genital pore, female; S, ventral shelf; VC, ventral cup.

Figs. 2 and 3.—Eggs of *Nudacotyle novicia.* P, polar filament; Op, operculum.

Fig. 4.—*Nudacotyle novicia* Barker, camera lucida drawing of stained and slightly compressed specimen, dorsal aspect. CP, cirrus pouch; ES, esophagus; Ex, excretory reservoir; GP ♂, genital pore, male; GP ♀, genital pore, female; I, intestine; Ov, ovary; OS, oral sucker; SG, shell gland; SV, seminal vesicle; T, testis; Ut, uterus; VG, vitelline gland; VD, vitelline ducts; VR, vitelline reservoir.

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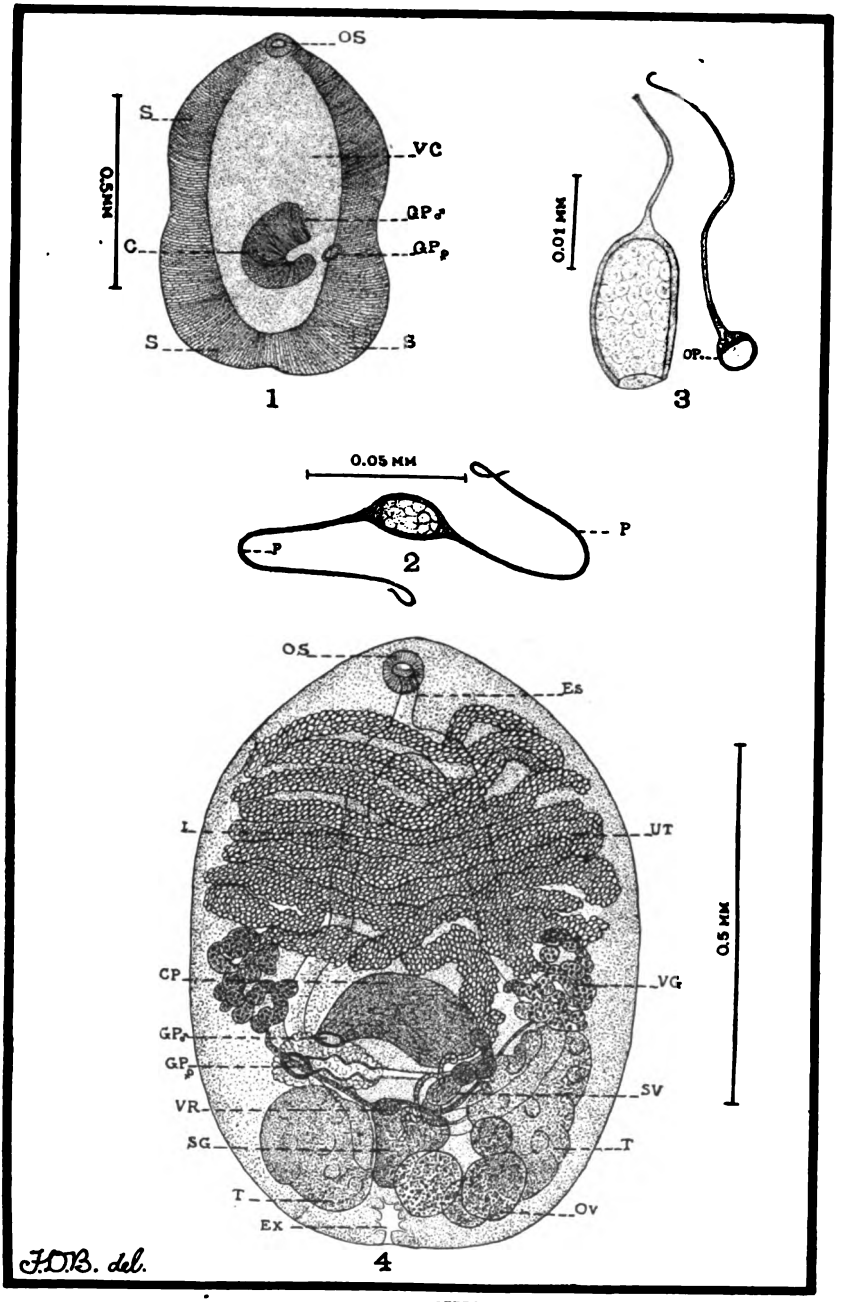

PLATE XXV

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DEPARTMENT OF NOTES, REVIEWS, ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress, without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated suggestions of suitable fields of investigation.—[Editor.]

SOME METHODS OF PREPARING INSECTS FOR DEMONSTRATION PURPOSES

In teaching entomology it is of considerable importance to provide the students with as much material as possible to aid them in identifying their specimens, to give them an adequate idea of the great number of insects available for study, and to impress upon them the value of insects for the demonstration of biological principles. It is therefore the custom to display upon the walls of the laboratory or on tables collections of insects which will serve these purposes. In the following paragraphs I wish to describe, with the aid of photographs, three methods that have been used in the entomological laboratory at the University of Michigan, which may be of interest to other teachers of the subject.

1. A method of displaying insects for purposes of identification. Photograph 1 shows a laboratory table, two feet wide and five feet long. On it is a removable frame which will carry six Comstock boxes inclined in such a way that their contents can be studied with ease. These boxes can be removed and others put in their place without difficulty. It is thus possible to display a large number of insects in a small space with the use of a laboratory table from which the frame that holds the insect cases can be removed and which therefore becomes available for other purposes. The framework above the boxes may be used for posting laboratory directions. notices, drawings, etc.

2. A method of displaying the life histories of insects. As is well known, an excellent method of displaying stages in the life histories of insects is to place the various stages in Riker mounts. These may be given to the students for study but may also be grouped in such a way as to make beautiful and instructive wall ex-

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hibitions. Photograph 2 shows a number of such groups, each group consisting of three or four mounts and arranged according to the economic importance of the insects represented, e. g., one group contains insects of the household (clothes moth, cheese skipper and carpet beetle) and another includes three shade tree pests (the horned tail, tussock moth, and leopard moth.) Students and visitors show considerable interest in these exhibits and, perhaps unconsciously, derive a great deal of information from them.

3. A method of displaying insect galls. Biologically the gall insects are among the most interesting of the whole class. The insect galls may be prepared for exhibition in the following manner (see Photo 3.) Racks three feet long, eight inches high, and two and one half inches deep are made, with the top piece hinged at both ends. Strips on the edges at the top and bottom prevent the bottles from falling out. Galls on stems are dried and placed in large mouthed vials about two inches in diameter, and a label is placed at the bottom. Galls on leaves are preserved in 10% formalin and placed in bottles about two and one half inches in diameter. The bottles or vials fit loosely enough in the racks so that they can be turned around and all sides of the galls can thus be examined but their removal is prevented by the strip near the top and bottom. If, however, it becomes necessary to take out a bottle, the hinge at one end of the top can be disjointed and the desired specimen removed. A background of white cardboard helps to bring out the characteristics of the galls. Such a rack as that described may, of course, be used for other material both zoological and botanical.

Zoological Laboratory, R. W. HEGNER.
University of Michigan.

THE SEDGWICK-RAFTER OCULAR MICROMETER AND ITS USES

In 1889 Prof. W. T. Sedgwick and Mr. George W. Rafter developed the so-called Sedgwick-Rafter method for enumerating microscopic organisms to be found in water,—a procedure which has since come into general use among biologists, chemists and engineers investigating or in charge of water supplies, and has been incorporated as a part of the Standard Methods of Water Analysis adopted

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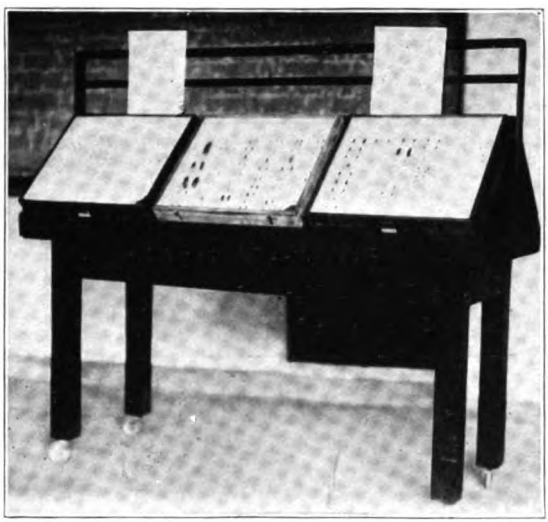

PLATE XXVI FIG. 1

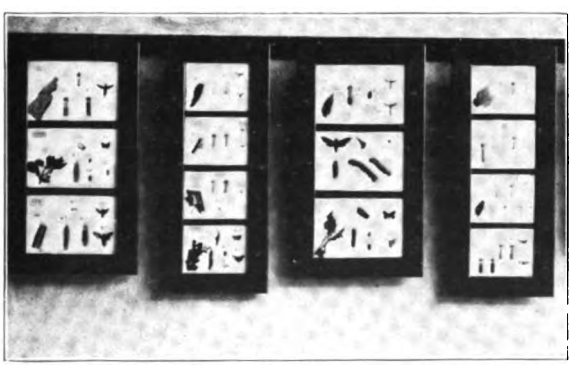

PLATE XXVI. FIG. 2

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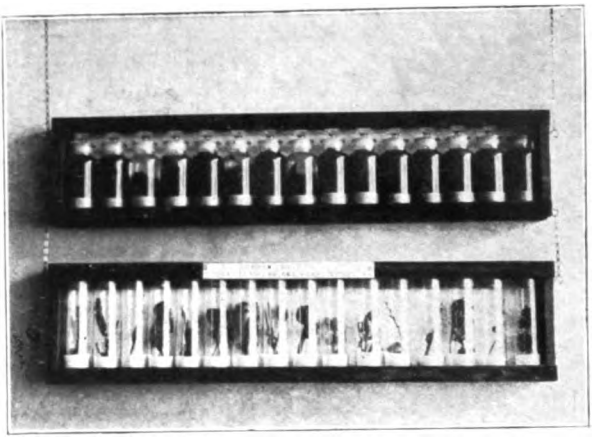

PLATE XXVII. FIG. 1

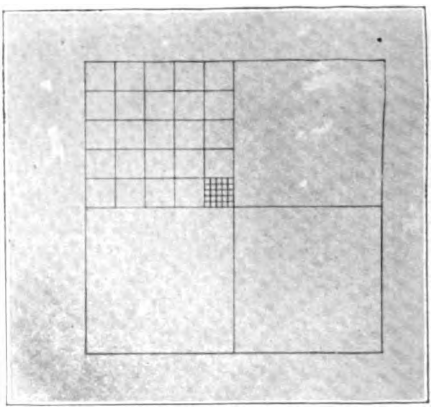

PLATE XXVII. FIG. 2

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by the American Public Health Association. This process, the details of which need not be mentioned here, involves the concentration of organisms by means of a sand filter and the microscopic examination of cubic millimeter volumes of the concentrate. The ocular micrometer which was devised for the purpose of ruling off a square millimeter surface on the "ringed" slide has been found useful not only in the enumeration and identification of organisms, but also in the broad field of general microscopic work and perhaps deserves a word of commendation to microscopists.

The micrometer is sub-divided as shown in the accompanying diagram and when used with an ocular of the No. 4-x Spencer type (2 inch) and a 16 mm. objective, can be so standardized by the aid of a stage micrometer that the large square will outline a square millimeter surface. The smallest square will then be 20 micra on a side and the micrometer will form a convenient measure for the larger microscopic objects. By only a slight change in the tube length, moreover, a standardization with the 4 mm. objective can be made which will increase the magnification five-fold, thereby reducing the side of the large square to 200 micra and that of the smallest square to four, so that, with the high power, the dimensions of all but the very smallest microscopical organisms can be readily obtained.

But ready adaptability to either high or low powers of the microscope is not the only commendable feature of this type of micrometer. There is a marked advantage in the way it is ruled into squares of various sizes. It enables one to measure the length and the breadth of an object at the same time and yet leaves the greater part of the field of vision relatively free from lines interfering with clear definition of objects. It is thus adapted to the use of the beginner as well as to that of the seasoned microscopist. The student beginning microscopic work in zoölogy, botany or histology, for example, is studying anatomy with no basis or standard of size-comparison, other than that offered by different objects in the same field. The standardization and use of the micrometer gives him an appreciation of the magnifying power of the microscope by demonstrating the apparent size of a familiar and fundamental unit, the square millimeter, and teaches him the approximate relative

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size of the microscopical linear unit or micron. The student finds the device convenient to handle, and easy to use and soon acquires a mental scale of unit areas by which he can estimate the size of organisms or other structures in micra much as he judges of macroscopic structures in centimeters or inches.

Ocular micrometers may be prepared either by engraving or photography and are not difficult to procure. Engraved micrometers are somewhat superior and are sold by Bausch and Lomb under the name of "Whipple's Eye-Piece Micrometer" at $3.50 each. Those made by photography are much less expensive and have been found entirely satisfactory. In either case, the actual size of the large square ruled on the glass should be 7 mm. on a side. In photography the lines are produced on lantern-slide glass by photographing a specially prepared sketch. The glass is then cut and mounted on a clean cover slip with gum damar. The only difficulty in using this type arises from the fact that the lantern-slide glass is rather thick and cannot be perfectly cleared without removing the fine lines. The fault is not serious, however, and may be readily overlooked if expense must be considered, for these micrometers can be made for about 75c each.* This is relatively very inexpensive, for the common linear ocular micrometer, which is much less adapted to work with living forms and hardly more valuable for exact measurements, is not obtainable for less than $1.25.

The advantages of this type of micrometer are its ready adaptability to general use with either high or low powers of the microscope; the definition of measured squares or unit surfaces, which are easier to use and to fix in mind than are linear units; a comparatively unobstructed "field"; and (in the photographic product) low cost.

*Unmounted ocular micrometers may be secured at this price from Mr. B. S. Turpin, 30 Trinity Place, Boston, Mass.

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FORMATION OF SPORANGIA IN STEMONITIS

Hilton (Jour. Q. M. C., Apr. 1916) contributes an interesting note on the method of forming sporangia in this common myceto-zoan. He had the good fortune to find considerable masses of plasmodium just in condition to observe the whole process. At noon the plasmodium formed somewhat rounded, solid, cushion-shaped masses. This surface differentiated in about a quarter of an hour into frothy, bubble-like hemispheres which divided and covered the entire surface regularly. By 4:00 P. M. each mass of hemispheres had contracted in width and increased in height; and the basal part constricted into flutings corresponding to the surface hemispheres. These flutings gradually contracted to pillars, the creamy protoplasm withdrawing more and more to the upper third. In another half hour black stalks were visible as the cores of the pillars. By 8:30 o'clock all the protoplasm had risen clear of the substratum and the still cohering heads of the sporangia appeared resting on a forest of black stalks. By 10:00 P. M. the sporangia had virtually assumed their permanent shape and were beginning to darken.

A DROUTH-ENDURING ZYGNEMA

Fritsch (Ann. Bot. 1916, pp. 135-149) reports upon a Zygnema especially adapted to terrestrial conditions. The longitudinal walls become much thickened, showing two or three successive layers. The outer layer of the wall is mucilaginous. This doubtless is an adaptation which prevents too rapid drying and aids resorption of water on return of moist conditions. It was found on Hindhead Common (England.)

There are two chloroplasts in the mature cell. Division takes place by an infolding or growth from the inner layer of the cell wall. The growth of this gradually constricts the protoplast, but the division may not be completed for considerable time. In this way the two daughter protoplasts have at first a connection thru the center of this plate.

With the oncoming of drouth the fat globules of the cell pass to the outer surface, and form there a dense layer just beneath the cell wall. When the plant begins to absorb water the fat drops

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come to be distributed again. When a drouth begins, the protoplast develops a new layer of the cell membrane. A cell divides not more than twice between two drouths. Agamic reproduction may take place early in the year, by the cell dividing unequally into a smaller pigment cell and a large, simple reproductive spore (akinete.) The pigment body disintegrates, the wall weakens and this becomes a breaking point for the dividing up of the filament.

BACTERIA AID IN FORMATION OF EUROTIUM

Sartory and Roger (C. R. Soc. Biol. Paris; 79: pp. 174-5) found, in a variety of *Aspergillus B* grown on damp straw, that they could secure promptly and abundantly and constantly the formation of perithecia, provided the culture was innoculated with microorganisms of the *B. mesentericus* group. Otherwise, he found, with pure cultures of the *Aspergillus* he could not get the *Eurotium* even with the aid of the various media hitherto suggested by students as valuable in this connection.

BACTERIAL INFECTION IN FRESH EGGS

Hadley and Caldwell (Bul. 164: R. I. State Col. Ag. Exp. Sta.) have discovered 8.7% of fresh eggs show bacterial infection of the yolk. The whites were sterile in all cases examined. The fertilization of the egg made no difference in the percentage. Forty different bacterial forms were found. There were no streptococci, and none of the groups causing hæmorrhagic septicæmia, enteritis, typhoid-dysentery, or diphtheria.

The study was instituted to throw light on the mortality of embryos in incubation, and the degree to which the mortality of chicks in brooders may be influenced by egg infection from mothers harboring the germs of diseases.

SOME REMARKABLE FEEDING ACTIONS OF AMEBÆ

Mast and Root (J. Exp. Zool. July, 1916) report studies of the capture of rotifers, paramecia, and other ciliates, by Ameba. They capture rotifers by flowing around the foot while attached. The protoplasm gradually flows upward along the stalk. The rotifer contracts in the effort to relieve the pressure; but when it extends

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the ameba begins its flow again. In the meantime the rotifer is gradually weakened by the digestion of the foot. It may require days to engulf the rotifer.

In the capture of paramecia the amebæ take a sort of mushroom shape. The free margin is made irregular by numerous short pseudopodia. This furnishes a space beneath the umbrella and recesses at the margin in which paramecia tend to come to rest. Pseudopodia enclose the paramecia from either side. In some instances pseudopodia reached only about half the length of the paramecium and by turning toward the body of the prey compressed it so as to cut the animal in two,—engulfing only the inner half. This whole process required only about ten seconds. This was not an isolated incident, but was observed many times.

The writers, by computation based on the amount of pressure required to cut paramecia with a thin glass thread, have reached the conclusion that this amputation of paramecia by Ameba could not be explained by surface tension in the protoplasm of Ameba.

CASE OF BROODING IN HOLOTHURIANS

Ohshima (Ann. Zool. Japon., June, 1916) reports a new case of internal brooding in holothurians,—*Pseudocucumis africanus*. As many as 25 and 27 young were found in the body cavity of the mother. Three such brood-carrying individuals were found. The author was unable to discover either how fertilization occurs or the young escape.

EFFECTS OF ACTIVITY ON NERVE CELLS

Kocher (J. Comp. Neur., June, 1916) after fifteen separate experiments, completely controlled, on six different species of animals, using cells from various regions of the nervous system, reaches the conclusion that there is no deviation from the normal, either qualitative or quantitative, that can be revealed by the most exacting cytological tests in nerve cells even in the most advanced fatigue. He ascribes the varying results of former investigators to:—(1) difficulty in separating the effects of normal activity from unavoidable shock or injury to the nervous system in killing the animal; (2) post mortem changes taking place before complete fixation; (3)

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varying chemical action of fixing agents; (4) solvent action of materials used in fixation and imbedding; (5) varying effects of chemical reaction between basic or acid stains and the different cell structures; and (6) effect of subjecting tissues to the temperatures necessary in manipulation. The author used an elaborate series of checks in control of the studies.

TRYPANSOME INFECTION IN MAMMALS

Lanfranchi (Atti. R. Ac. Linc. 1916, pp. 369-73) believes that *Trypanosoma brucei, T. gambiense* and *T. vodiense* can pass from the blood of a pregnant mother into the milk; and that by the first two, at least, the fœtus may also be infected in the same way.

IMPROVING TECHNIC FOR SHOWING DETAILS OF DIVIDING CELLS

Allen (Anat. Rec., July, 1916), as the result of a series of critical experiments to perfect the technic for demonstrating the details of mitosis in the central nervous system and in the testis of the albino rat, without producing distortion of adjacent tissues, offers the following suggestions:—

"For cytological work, the slightest gain at any point in the technic is worth working for.

"Very gradual changes of fluids, agitation of fluids during changing, and slow infiltration appear essential in order to get the best results from any fixative.

"The addition of a low percentage of urea to fixing fluids results in sharpening the chromosomes and preserving the structure of the achromatic nuclear material. It may help the penetration of the fluids.

"Picro-formol-acetic mixtures are more effective when used at about 38° C. Cold is detrimental.

"Flemming's fluid is more effective if used at 0° C. or a few degrees lower.

"Flemming's fluid is of no value as a brain fixative at any temperature. At times (if urea is added) it isolates metaphase and anaphase chromosomes in spermatocytes somewhat better than any

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other fixative tried (except B-15*), but is hard on the rest of the tissues and shrinks heavily.

"Anilin oil is an excellent substitute for the higher alcohols.

"Xylol shrinks tissues more than the vegetable oils."

VISUAL EFFICIENCY IN THE USE OF OPTICAL INSTRUMENTS

Purkis (J. R. M. S., June 1916) makes some good practical working suggestions for those who use the microscope for prolonged observations, where necessity of accurate observation and minimum fatigue are necessary.

1. The fact that only one plane is in sharp focus at a time and that other planes show dimly tempts the novice to strain the eye in the effort to make it see things which are not clearly in focus, instead of adjusting the distance.

2. This suggests also that care should first be taken to discover the limitations of the instrument which cannot be corrected by manipulation and to accept these, refusing to try to make the eye compensate for these limitations, by intensely close observation.

3. The eye should look at the field almost casually. What it cannot see by looking quietly at the object, it cannot see by an intense and strained gaze.

4. Recognizing that there is a certain amount not only of shock to the eye in sudden changes from dark to light luminosities and conversely, but also strain of the eye in the effort to see well before the re-adjustment is completed, it is important to avoid so far as possible sudden changes of luminosity. In this connection it is well (a) to see that the illumination of the room is not in too great contrast with that of the instrument; (b) to modify luminosity to compensate when passing from one objective to another; (c) to avoid sharp contrasts of luminosity within the field itself; and (d) when resting the eye preparatory to observation or during observation to do so in approximately the same illumination to be used in the field of the instrument.

*B-15 is a picro-formol chrome-acetic mixture in urea:

Picric acid	75cc
Formol	25cc
Acetic acid	5cc
Chromic acid (crystal)	1.5 grams
Urea	2.0 grams

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NORTH AMERICAN DIATOMACEÆ

A new guide for the American student of Diatoms will be of interest to many members of the American Microscopical Society. Charles S. Boyer has issued a monograph of most painstaking character on the Diatomaceæ of Philadelphia and vicinity. The book opens with an introduction descriptive of the physical condition of the Philadelphia region,—which means a territory with a radius of about 100 miles from Philadelphia. A short chapter is devoted to the structure, reproduction, evolution, and rôle in nature of the diatoms.

The body of the book is igven to keys, descriptions, and figures of nearly 600 species and varieties. There are 700 drawings by the author illustrating these species. All these drawings are to uniform scale,—about 800 diameters. In his classification the author adopts Schuett's synthesis of the various bases of classification of Pfitzer and Petit, Smith, Mereschkowsky, as well as the older methods. The synonymy is for the most part omitted.

The appendix contains suggestions as to the collection and preparation of diatoms for mounting and study. Many of these will prove most helpful for the inexperienced student of the group. The headings:—Collection of fresh water material; Collection of marine material; Blue clay deposit; Cleaning material; Preparation of strewn mounts; Preparation of selected mounts; Instruments required,—will sufficiently indicate the scope of these hints. Mechanically the book is very attractive. It is bound in art vellum cloth, the pages are 9x12, and the text is printed on heavy unglazed paper.

The Diatoms of Philadelphia and Vicinity, by Charles S. Boyer, A.M., F.R.M.S., 143 pages, 700 illustrations. J. B. Lippincott Company, Philadelphia, Pa., 1916. Price $5.00.

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NECROLOGY

Burrill, Thomas J, Ph.D. '78, President A.M.S., 1886 and 1904.....Urbana, Ill.

Lewis, Ira W. '87.....Dixon, Ill.

IRA W. LEWIS

Mr. Lewis, who was for nearly twenty years a member of the American Microscopical Society, died May 26, 1915, at the age of 73. He was a man of most substantial character and attainments. For 45 years he was connected with the office of the Clerk of the Circuit Court of Lee County, Ill., as Deputy or as Circuit Clerk. The Bar Association of the County testified in a public Memorial Service to his unvarying efficiency and high mindedness.

He was a devoted churchman and gave much of his energy to work in the moral and religious education of youth. He was a self cultured man, and his memorialists testify with great uniformity that he was one of the most remarkable and versatile men of his community.

In the meantime he gained local note as a student of books and of nature. He was very devoted to his work with the microscope and collected an extensive library of microscopical journals and scientific books. It would be greatly to the credit of American culture if we had more such amateur students with the microscope.

His wife survives him.

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ORGANIZED 1878 INCORPORATED 1891

OCTOBER
1916

VOLUME XXXV No. 4

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Attention of members of the

AMERICAN MICROSCOPICAL SOCIETY

is called to the announcement of Grants
from the Spencer-Toller Research Fund.
(See TRANSACTIONS, July, 1913)

These awards are to stimulate and aid research in Microscopic work

1. **The Grantee must be a member of the Society**
2. **Resulting discoveries must first be offered for publication in the Society Transactions.**
3. **Application should be made to Dr. Henry B. Ward, Chm., Urbana. Ill.**

NOTICE TO MEMBERS

The Secretary will consider it a favor if members will notify him of non-receipt of numbers of the Transactions, and of changes of address.

T. W. GALLOWAY,
Secretary-Editor.

Beloit, Wisconsin.

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TRANSACTIONS

OF THE

American Microscopical Society

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The Society does not hold itself responsible for the opinions expressed by members in its published *Transactions* unless endorsed by special vote.

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Notice to Members

Business Sessions of the *American Microscopical Society* will be held at New York City, as follows:

Dec. 28 (Thursday), 8:15 A. M., Executive Committee will meet at breakfast at *Hotel Martinique.*

Dec. 28, 4:30 P. M., General business meeting of the Society, immediately following the adjournment of the American Society of Zoologists, in the Hall used by them.

The Headquarters of the *American Microscopical Society* will be the Hotel Martinique, Broadway, 32-33d Sts. It is convenient—near the Pennsylvania Station—and reasonable in rates.

T. W. Galloway, *Secretary,*
Beloit, Wisconsin.

(This Number was issued on December 16, 1916.)

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TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Installments)
Vol. XXXV OCTOBER, 1916 No. 4

REPORT OF THE SECRETARY AND EDITOR

T. W. GALLOWAY
Beloit College, Beloit, Wis.

Three years ago the Secretary reported to the Society the outstanding facts relative to the state of the organization during the first term of his service. The present number marks the close of the second term of three years. In the absence of large and well attended meetings of the members, where discussions both of scientific and business details would place in the minutes much that would enlighten the members as to the work of the Society, it appears that a triennial report by the Secretary may well supplement the annual reports of the Treasurer and the Custodian.

It seems necessary, for the sake of efficiency in such a Society, that a large amount of the leadership and decision of policies fall upon the Secretary. The organization pays for this freedom of action on his part, however, in a certain loss of responsibility and mutuality on the part of the membership. The Secretary feels very strongly the lack of positive and constructive,—or for that matter any kind of,—criticism.

Attention was recalled in the last report to the transitional period through which the Society has passed in the last quarter of a century, and to the decision four years ago to try to find a function for the Society which would be at once worthy of its best traditions and place it in a position in which it may continue, in spite of the multiplication of special societies, to serve American science and scientists. It is too much to hope that this has been done as well as it might have been done. Yet evidences are not wanting that the policy adopted then has been on the whole well chosen.

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There is, however, one group of our most faithful and effective members whom we have not been able to serve as the Secretary would like. This is the group of students who are working with the microscope independently, usually out of contact with colleges and laboratories, browsing more or less in the numerous interesting fields opened to them by the microscope. In many cases they are working in an amateur way, more or less intensively upon one or more of the groups of microscopic forms. In some instances these students are serving, by private correspondence, as a clearing-house for information for amateurs. The Secretary has the impression from correspondence that this group is again becoming larger than it has been for 25 or 30 years. It finds our research articles too technical and would greatly prize a much enlarged discussion of elementary and advanced, up-to-date methods of manipulation, such as distingushed the "Journal of Applied Microscopy" some 15 years ago. A department devoted to these interests would probably find acceptance with a considerable number of people. Two English journals,—the "English Mechanic and World of Science" and the "Journal of Micrology and Natural History Mirror" serve this end in some measure. The Secretary-editor has tried, in an experimental way, to relate the Society to some of the local amateur microscopical clubs with the hope that at least a small department of this type of material might be established in the *Transactions* to encourage again in America high grade amateur work on the part of intelligent people who do not expect to use the microscope for professional ends. Thus far we have been unable to do anything significant in this direction. All his own spare time is so taken up in carrying out the policy adopted as our main objective that he could not, even if his ability ran in this direction, do the work himself. It has not been possible to find any one at once able and willing to do this in the large and scientific way which would be necessary to make it worth while. Suggestions from the members will be welcome.

In other respects the work of the Society seems to have progressed reasonably. The usual volume of four numbers and about 300 pages has been published each year. It is still true that we do not receive contributions enough in the varied fields of interest

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to make it possible for us to select articles in such a way as to furnish a balanced biological ration. For some years the zoological contributions have dominated. Thanks to our botanical friends, chief among whom was Professor Bessey, a much larger botanical element has appeared in the *Transactions*. For various reasons it has been impossible to issue the quarterly promptly in the month of its date.

The Secretary has been particularly disappointed in respect to one class of contributions which he hoped to feature in the *Transactions*. It is a strong conviction of his that nothing we can do would serve American science so distinctively and give the *Transactions* so welcome a place in every biological library as a series of digests covering part by part the special fields that go to make up biology. If this territory were marked off into 20 or 25 subdivisions, and an expert were to present once in five or six years a thorough-going summary of each of these, we could by giving one such digest in each issue cover the field in this time. Such a digest would not undertake to be exhaustive as might be needed by the men in the field; but would be interpretative of the most important results, the main conclusions and tendencies and prospects, together with such references to the important papers as would serve the need of the student outside the particular field rather than of those within it. A few such have appeared and these in every way have confirmed the editor in his conviction of the tremendous service such an enterprise would render to the rank and file of American students and teachers of biology. The attempt has thus far failed because the men who are most able to command both the territory and the audience are unable to command the time. The Secretary is coming to be of the opinion that the income of our Spencer-Tolles Fund apportioned as an honorarium for digests of this kind would render a more distinctive and a more vital service than it can do as our present rules demand.

Several suggestions have come urging that emphasis be placed upon the technic and methods of biology and microscopy. Some progress in this direction is being made and more is planned. We have not yet secured a steady stream of such communicatlons; but

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there has been an encouraging response. The Secretary urges strongly that every member who sees this notice "highly resolve" to supply this department with a description, illustrated if desirable, of every new unpublished device for investigation, illustration, and teaching which his own experience approves; and furthermore secure similar returns from all his colleagues, whether members of this Society or not. There is no present point at which so vital a service can be rendered the *Transactions* and the membership by the individual member. We shall be glad to become a clearing house for the best biological methods developed in America.

We are still growing in numbers and in financial strength. When the present Secretary took charge in 1909 there were on the roll 226 names of members and 33 subscribing libraries and individuals. A considerable number of these quickly dropped out. In all, 127 of this list have ceased to be members, leaving 99 members at present whose connection with the Society goes back of 1911. In the six years there have been added 300 members and 67 subscribers. Of these members 57 or about 20% have discontinued. Of the subscribers only three or four have been lost. There is now an enrollment of 343 members and 97 subscribers, or a total of 440 supporters. One of the chief deductions from our membership history is that the list of subscribing libraries should, if possible, be run up to 150 or 200. This would furnish a group of supporters not nearly so fluctuating as individual memberships. A library once possessed of a fairly complete file of our volumes is increasingly likely to continue its connection. Because of this the Executive Committee at the Cleveland meeting authorized the Secretary to adopt with libraries a liberal policy in the distribution of partial sets made up of those back volumes of which we have excessive numbers. We have been able to supply partial sets, sometimes running as high as 18 or 20 volumes, on condition that the library would begin a subscription with Vol. 29, at which point we began to publish in parts. The Secretary will welcome correspondence from members connected with institutions with growing and permanent libraries, which may not be

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able to buy a complete set, relative to the conditions of this distribution of the back volumes.

The total resources of the Treasurer in 1911 were $782. The annual resources for the succeeding years were $1234, $1433, $1379, and in 1915, $1522. The society is very nearly to a point of self-support, which will not demand a high-pressure campaign for members, but merely demand effort enough to replace those who are lost. It ought to be possible within the next year or two to push the members and subscribers to the 500 mark.

The Spencer-Tolles Fund under the wise and enthusiastic management of the custodian, Mr. Pflaum, has climbed steadily during the period, from $3352 in January, 1912. The custodian will report at the next meeting close to $5000. This will mean an annual income of $300,—which may be devoted to the advancement of science. The Secretary urges those members of the Society who are engaged in research, in any field where such a grant would signally advance the investigation, to make inquiry of the Chairman of the Committee on Grants,—Professor H. B. Ward, University of Ill., Urbana, Ill.

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A COMPARATIVE STUDY OF EPIGYNY IN CERTAIN MONOCOTYLEDONS AND DICOTYLEDONS*

By MARGARET HANNAH

It is not the purpose of this study to give the complete details of the organography of the different flowers, which have been chosen for this comparison but the steps in the development of the floral organs have been given in sufficient detail to show the development of the inferior ovary. From these a comparison of epigyny in the two great divisions of the flowing plants has been made. Very little work has been done on this special phase of the subject, although different writers have their own explanation for the manner in which epigyny is produced.

Martin (6) in 1892 wrote, "The real origin and behavior of the floral organs in their younger stages of development as correlated with the inferior ovary has attracted but little attention, and therefore, no definite statement can be made as to the true relationship existing between the floral organs in their embryonic condition". In the same paper, in speaking of the tubular mass of tissue, he wrote, "In which there is a complete fusion of the parts until liberated", and a theory was proposed "that all the floral organs are coalesced in their initial state in the annular wall, and each appears as the upper parts are liberated".

Gray (5) wrote, "Where the adnation is complete to the top of the ovary and none beyond it".

Goebel (3) in 1887 defined an epigynous flower in this manner, "The walls of the ovary are formed from the torus itself, which is hollowed out into the shape of a cup, or even a long tube, while the carpels which form the entire wall of the free superior ovary, spring like the perianth and androecium from the margin of the hollow torus and only close its cavity above, being there prolonged into the style and bearing the stigma. The inferior ovary is formed by the terminal growth being retarded and by the outer rim growing upward. The placentae may be regarded as the margins of the carpels running down the inner surface of the ovary wall".

*Contribution from the Department of Botany, The University of Nebraska.

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In his Organography of Plants, published in 1901, Goebel (4) said in contradiction to what he had said in his book, published in 1887, "On account of deficient historical investigation, the view was formerly advanced, that the ovary of the epigynous flower is formed from the cup-like flower axis, and the carpellary leaves only produce the style and stigma. Comparative morphology has rightly contradicted this interpretation, which is still found in many books. As the history of the development shows, the carpels share in the formation of the ovarian cavity, and the ovules have no other origin than in the superior ovary.

"In all inferior ovaries, the vegetative point becomes at an early period more or less concavely hollowed out, and the leaf structures of the flower arise, partly from the margins, and partly from the inner surface of the depression. Whether one describes the marginal part of the flower axis as a 'congenital concrescence' of the different leaf whorls of the flower is an arbitrary matter, because the flower axis ends its active existence with the bringing forth of the leaf structures of the flower. The earlier the flower axis assumes the cup-like form, the more will we in general ascribe its character to the flower axis. The later this form is assumed the more will its features approach the more primitive condition as we find it in hypogynous flowers".

Wylie (8) in 1904 worked out the morphology of *Elodea canadensis*. In this study, he showed how epigynous flowers have developed in this primitive type of monocotyledon.

Coulter (1) in 1883 investigated the dandelion flower, giving as his purpose an investigation of the development of the inferior ovary. He said, "The inference is that all four of the floral organs are blended together in the primitive ring, which rises from the original obconical mass, that they are essentially hypogynous and that their separate appearance is a freeing of their upper extremities. It was attempted in vain to detect in the primitive ring or later in the wall of the ovary any evidence of the blending of two or more distinct parts. No such indication could be found, and the inference that all four floral parts are represented in the wall of the inferior ovary rests, not so much upon the structure of the wall, as upon the order of the succession in the appearance of the floral parts".

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He believes the theory, that the primitive ring belongs to the receptacle, is not tenable for two reasons: (1) "the late appearance of the calyx"; (2) the fact that the corolla lobes appear with the ring and not after it, indicating that the ring belongs to the floral organs. "The inferior ovary is produced by an arrest of the development of the floral axis the rising in a peripheral ring of the floral organs and a gradual arching over of the carpellary leaves".

Merrell (7) in his paper on Silphium said, "The outline of the receptacle soon became angular by the upward growth of the marginal ring, which is the beginning of the corolla tube".

Six species of Monocotyledons and nine species of Dicotyledons, representing twelve different families, have been included in this comparative study.

The different species are named below under their respective groups:

1. Monocotyledons: *Sisyrinchium augustifolium; Gladiolus gandavensis; Iris germanica; Freesia refracta, Var alba; Musa sapientum;* and *Canna indica.*
2. Dicotyledons: *Malus ioensis, Ribes aureum, Fuchsia speciosa, Citrullus vulgaris, Sanicula canadensis, Galium aparine, Sambucus canadensis, Valeriana officinalis* and *Helianthus annuus.*

Monocotyledons. Plates XXVIII-XXXI

Sisyrinchium angustifolium. Plate XXVIII

The flowers are produced in clusters, developing in centripetal order. The buds begin as protuberances of meristematic tissue, which appear slightly triangular in cross section, due to the arrangement of the flowers in the cluster. Soon three lobes, the beginning of the stamens, grow up from the rim of the receptacle, which has become flattened (Plate XXVIII, Fig. 2). The lobes of the perianth soon appear at the sides of the mass (Fig. 3). At about the same time, the tissue just below the origin of the perianth and the stamens begins to elongate, forming a tubular ring about a shallow cavity in the center (Figs. 4, 5 and 7). The three lobes, which are to form the carpels, soon grow out from the inner

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rim of the tubular cavity (Figs. 5 and 6). A mass of tissue soon roofs over the central cavity, and a continuation of the three lobes upwards forms the style branches (Fig. 12). From three sides of the cavity, tissue begins to grow towards the center (Fig. 8). The fusion of these three outgrowths divides the ovary into three parts (Figs. 10 and 11). The tissue at the point of origin of the sepals and the petals grows *en masse,* so that the sepals and petals are joined together for a short distance, forming a tubular ring.

Gladiolus gandavensis. Plates XXVIII and XXIX

The earliest stages in the development of the flower of the Gladiolus were not found, but stages showing the growth of the tissue forming the tubular rim and the central cavity are shown in Plate XXVIII, Fig. 13. The perianth and stamen lobes have appeared. Further development is similar to Sisyrinchium. The figures show the origin of the parts and the final development of the inferior ovary. The order of the succession of the floral leaves is sepals, petals, stamens and pistils.

Iris germanica. Plate XXIX

The development of the flower of the Iris is almost the same as that of Sisyrinchium angustifolium, differing in the order of the succession in the development of the floral leaves. In the Iris, the order is perianth, stamens, and carpels (Figs. 2, 3 and 4). The petals and stamens appear almost at the same time, so nearly so, that it is difficult to determine which lobes really appear first. In Sisyrinchium, the stamen lobes grow out first (Plate XXVIII, Fig. 2) and the lobes of the perianth grow out at the sides, as described under Sisyrinchium and shown in (Plate XXVIII, Fig. 3.)

Freesia refracta. Plates XXIX and XXX

The flowers are produced on two sides of an elongated axis, each flower subtended by a bract. The flower begins as a protuberance from the side of the flower axis. This undifferentiated mass of cells soon flattens and broadens, and distinct lobes appear, the sepals (Fig. 7). Inside the whorl of sepals, the stamens ap-

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pear (Fig. 8). At this time, the tissue below the lobes begins to elongate, forming a tubular ring with a shallow cavity in the center (Fig. 9). The lobes of the carpels appear (Fig. 1 and 2, Plate XXX). There is further elongation of the tissue just below the point of origin of the floral organs. The three carpel lobes grow out into the central cavity, and, growing upward and toward the center, cover the cavity. An upward extension of the three masses forms the three style branches, which appear as three lobes only at the top (Fig. 3).

Musa sapientum. Plate XXX

The method of origin and early development of Musa sapientum is like the Iris, Freesia and Gladiolus. The later development shows a zonal development of the tissue near the bases of the sepals and petals, so that the sepals and two of the petals are joined together nearly to their tips. The other petal remains free.

Canna indica. Plates XXX and XXXI

The flower begins as an outgrowth which broadens; the sepals grow out from the top of this undifferentiated mass (Fig. 11, Plate XXX), and just inside of these lobes, other masses grow out. and very soon separate into two parts (Plate XXXI, Fig. 1). The inner whorl forms the four staminoidia and the one fertile stamen, and the other whorl the petals. The tissue at the base of these lobes now elongates in the peripherial portion leaving a hollow central cup (Figs. 2 and 3). Further devolpment resembles Sisyrinchium, differing in the form of the style. The tissue at the upper portion of the central cavity grows up into a somewhat flattened mass forming the style (Figs. 4 and 5).

Wylie (8) found the development of the primitive, Monocotyledon, *Elodea canadensis,* quite like that given above for the higher Monocotyledons. Buds begin as protuberances, apex of receptacle flattens and broadens, a mass of tissue grows up leaving a tri-radiate slit down the center. The order in Elodea is sepals, three sterile stamens, three stigmatic lobes, and finally the petals.

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In the Monocotyledons described above the tri-radiate slit did not form until after a circular cavity formed and three masses of tissue grew out into this cavity.

Dicotyledons. Plates XXXI-XXXV

Malus ioensis. Plates XXXI and XXXV

Material was not collected early enough to show the first stages in the development of the flower of the Malus, but early stages to show the development of the inferior ovary were found. The floral organs, sepals, petals and stamens are shown in Plate XXXI, Fig 6. At the time that the lobes of the floral organs appear, there is an upward growth of the outer rim of the receptacle, leaving a shallow, broad, central cavity (Fig. 7). The carpel lobes grow out from the bottom of this shallow cavity (Fig. 8). The elongation of the rim of this cup produces the wall of the inferior ovary (Fig. 9). Very soon after the appearance of the lobes of the carpels, a cross section of the ovary shows five masses of tissue growing in toward the center (Fig. 10).

Ribes aureum. Plate XXXII

The order of the development of the floral organs of Ribes is similar to that of Malus. The elongation of the cylindrical mass of tissues leaves a narrow slit (Fig. 3), from the sides of which, two protuberances grow out, forming two opposite lateral placentae (Figs. 4, 5 and 7). Two lobes of the style form with only one cavity in the ovary. The cross section shows two bundles, one for each carpel (Fig. 7 d).

Fuchsia speciosa. Plates XXXII and XXXIII

The flowers are solitary in the axils of opposite leaves. The flower buds begin as hemispherical masses of undifferentiated tissue (Plate XXXII, Fig. 8a). The receptacle flattens, and the four lobes of the sepals appear on the peripheral portion. This outer portion elongates, forming a shallow cup-shaped cavity in the center (Figs. 9 and 10). The petals and stamens grow out from the inner surface of this cup, near the bottom (Fig. 11).

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The zonal development continues. The cup-like cavity elongates. Just below the stamens, the four lobes of the carpels appear (Fig. 12). An upward prolongation of these lobes forms the style branches and roofs over the central cavity (Fig. 13). The four cavities of the ovary are formed by four masses of tissue growing toward the center and fusing. (Plate XXXIII, Figs. 1, 2 and 3).

Citrullus vulgaris. Plate XXXIII

The origin and development of the floral organs of the flowers of Citrullus are very similar to those of Fuchsia, except for the number of parts. There are the five sepal lobes (Fig. 6), petals (Fig. 7) and stamens (Fig. 8). As in the other Dicotyledons, there is the hollow cavity in the center (Fig. 9). The sides of the cup elongate to form the inferior ovary (Fig. 11).

Sanicula canadensis. Plates XXXIII and XXXIV

What has been said about the Fuchsia is also true, in part, for Sanicula. From the flattened receptacle the five lobes of the sepals grow out (Fig. 13). The petals appear just inside but alternate with the sepals (Fig. 13). At the point of origin of these parts there is the zonal elongation, so that the sepals and petals are adnate a short distance above the ovary. After the cavity of the ovary is formed, a two-lobed mass of tissue grows up from the bottom of the cavity (Fig. 15). The base of this tissue elongates carrying the lobes upward, until they meet the tissue, which roofs over the cavity of the ovary Plate XXXIV (Figs. 1 and 2).

Galium aparine. Plate XXXIV

The petals are the first of the floral organs to appear in Galium (Fig. 4). The sepals are late in appearing, and then do not develop very far (Fig. 5). Just below the stamens the stigmatic lobes appear and between these a convex mass grows out from the bottom of the cavity. This mass soon becomes two-lobed (Fig. 6), enlarges (Fig. 7), and develops two sporangia with their integuments (Fig. 8). With the appearance of the stigmatic lobes and the sporangia, there is an elongation of the

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tissue at the base of the sporangia. This upward growth and a downward growth from the roof tissue divides the central cavity into two separate locules (Figs. 9 and 10).

Sambucus canadensis. Plate XXXIV

The origin of the floral organs is the same as the others described. The sepals grow out from the edge of the flattened mass of tissue (Fig. 11). The petal lobes appear (Fig. 12) while the receptacle is still flat (Fig. 13). The sepals elongate and enclose the other parts. Figure 14 shows the appearance of the stamens. The central cavity flattens out, so that it becomes broad and shallow (Fig. 15). The carpel lobes appear as outgrowths from the bottom of the cavity (Fig. 16). This upward growth coalesces with four lobes growing in from the inner surface of the cavity. Tissue grows down from above and meets the upward growing tissue (Figs. 1 and 2, Plate XXXV).

Valeriana officinalis. Plate XXXV

The five lobes of the petals appear at the edge of the flattened receptacle (Fig. 4). The lobes on one side of the flower grow more rapidly than the others, forming a slightly zygomorphic flower. A single stamen grows out (Figs. 6 and 7). At the same time, there is an upward growth of the tissue at the base of the petals and the stamens (Fig. 7). The sepals grow out from the outer surface of the tubular ring, and the carpel lobes form near the bottom part of the inner surface of this ring. There are then two rapid zonal elongations, one of the tissues growing up to form the ovarian cavity, and the other the tissue at the base of the sepals and stamen. The petals form a tube and the stamen is joined to the petal some distance above its point of origin.

Helianthus annuus. Plate XXXV

The details of the development of the flowers of Helianthus are essentially like those for Valeriana. The order of the succession of the parts is petals, stamens and carpels (Figs. 10-16). The sepals appear at about the same time as the carpels. There

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are the two zonal elongations; one forms the tubular corolla, and the other the wall of the inferior ovary.

Summary

In conclusion, some of the points observed in the study of the development of the inferior ovary in the above species of Monocotyledons and Dicotyledons are summed up in the following comparisons of the two groups:

1. The flower buds of all originate in the same manner by a portuberance of undifferentiated, meristematic tissue growing out from the flower axis. This mass flattens and broadens, and several whorls of lobes appear at the upper outer edge.

2. The order of the succession in the development of the floral parts is not the same for all the genera of the Monocotyledons, nor for all the Dicotyledons. The order, sepals, petals, stamens and carpels is found in all the Monocotyledons except Sisyrinchium. The same order is found in all the Dicotyledons, except Helianthus, Galium and Valeriana. In these the order is petals, stamens, sepals and carpels. In Sisyrinchium, so far as could be determined, the stamens appeared first.

3. In none of the cross sections could there be found any evidences that several floral parts had begun as separate parts and then joined together.

4. In both groups, there is evidence of adnation of parts, but this union of parts is formed by a zonal elongation of the tissue at the point of origin.

5. An examination of one similar stage of development, through the whole series of plants studied (the stage chosen was the one showing the appearance of carpels, Plate XXXVI), shows one difference between the Monocotyledons and Dicotyledons. In the Dicotyledons the lobes of the carpels grow out from the bottom of a very shallow cup, while in the Monocotyledons the carpel lobes push out from the sides of a slightly elongated cup.

6. After the appearance of the carpel lobes the annular rings in all elongate, forming the inferior ovary. In some cases, the tissues above the ovary also elongate, so that the stamens appear to branch out from the petals, and in others the petals and sepals are joined.

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The differences of opinion as to the origin and development of epigyny seem to be based on the question, whether inferior ovaries are formed by a coalescence of all parts until they are liberated above, or whether the receptacle grows up in the form of a cup with the lobes of the sepals and petals coming from the rim. After making a careful study of the different plants described above, it seems that epigyny in both the Monocotyledons and the Dicotyledons develops in practically the same manner. This appears to be by a zonal elongation of the tissues just below the point of origin of the floral leaves. This elongation may begin at the appearance of the first lobes or it may not begin until the lobes of all the parts have appeared.

The origin and development is similar, with the one exception given under 5 above, whatever theory as to the nature of the wall of the ovary, is accepted, whether it has the character of the floral axis or the character of the floral leaves.

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EXPLANATION OF PLATES

The lettering for all the figures is the same:

se = sepals	pl = placenta
pe = petals	s = stamens
p = perianth	st = style and o = ovules

PLATE XXVIII

Figs. 1-12 *Sisyrinchium angustifolium* (X 68.7).

Fig. 1. The young stem tip on the left, a, young flower bud; b, bract.

Fig. 2. Longisection of a young flower bud, showing the beginning of the lobes of the stamens.

Figs. 3, 4, 5, 6 and 9. Longisections thru the center of young flower buds, showing the origin and growth of the sepals, petals, carpels and ovary cavity.

Figs. 7, 8, 10, 11. X—sections of ovary, showing central cavity in Fig. 7; the development of placenta and ovules in Figs. 8, 10, and 11; Fig. 7 about same stage as Fig. 4; Fig. 10 same as Fig. 9 and Fig. 11 as Fig. 12.

Fig. 12. Longisection of an older bud, showing the inferior ovary.

Figs. 13-15. *Gladiolus gandavensis* (X 68.7).

Fig. 13. Longisection of flower bud, s, beginning of stamen; p, perianth; b, bract.

Fig. 14. Older stage of same.

Fig. 15. Shows the beginning of carpels, c.

PLATE XXIX

Fig. 1. *Gladiolus gandavensis* (X 23.2) se, sepals; pe, petals; st, style.

Figs. 2-6. *Iris germanica.* Figs. 2, 3, 4 (X 68.7). Figs. 5 and 6 (X 38.7). All are longisections of young flowers, cut near center of bud.

Fig. 2. Young flower bud; p, perianth; b, bract.

Fig. 3. s, beginning of stamens.

Fig. 4. Further development; c, carpels.

Figs. 5 and 6. Later stages of same.

Figs. 7-10. *Freesia refracta* (X 68.7). Longisections of young flower buds. s, stamens; perianth; se, sepals. Figures numbered in order of the appearance of different parts.

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PLATE XXX

Figs. 1-3. *Freesia refracta.* Figs. 1 and 2 (X 68.7).
Figs. 1 and 2. Longisections of the same flower. Fig. 1 cut thru the center and Fig. 2 at one side of the center.
Fig. 3. (X 38.7). Longisection of an older flower.
Figs. 4-9. *Musa sapientum* (X 68.7).
Fig. 4. Young flower bud.
Fig. 5. Longisection of an older flower bud.
Figs. 6-9. Longisections, showing origin and growth of parts as lettered.
Figs. 10 and 11. *Canna indica* (X 68.7).
Fig. 10. Longisections of three flower buds, a; b, bracts.
Fig. 11. L-section of one older flower. s+pe, stamens and petals; se, sepals.

PLATE XXXI

Figs. 1-5. *Canna indica.* Figs. 1-3 (X 68.7); Fig. 4 (X 19.3).
Figs. 1, 2, 3 and 4. L-sections, numbered in order of development of parts and lettered as above.
Fig. 5. Cross section of pistil (X 19.3). a, cut near top of style; b, near center; c, below b.
Figs. 6-10. *Malus ioensis* (X 31.2).
Figs. 6, 7, 8, 9. Longisections, showing the origin and development of the parts of the flower.
Fig. 10. Cross section of a young ovary. pl, placenta.

PLATE XXXII

Fig. 1. *Malus ioensis* (X 18.7). Longisection of a flower, the inferior ovary well developed.
Figs. 2-7. *Ribes aureum.* Figs. 2-5 (X 68.7); Figs. 6 and 7 (X 31.2).
Figs. 2, 3 and 6. Longisection of young flower numbered in order of development.
Fig. 4. Cross section of the ovary of Fig. 3.
Fig. 5. X-section of an older stage.
Fig. 7. X-section of pistil, stage same as Fig. 6.
a, cut at dotted line "a"; c, on dotted line "c"; and b, cut between "a" and "b"; d, cut thru ovary, shows young ovules.
Figs. 8-13. *Fuchsia speciosa.* Figs. 8-10 (X 68.7). Figs. 11-13 (X 31.2).
Fig. 8. Longisection of young stem tip.
a, a, young flower buds on either side of stem tip.
Figs. 9 and 10. Development of sepals.
Figs. 11, 12 and 13. Appearance of sepals, petals, stamens, and carpels. Dotted lines in Fig. 12 show where petals will appear as slide is moved along.

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PLATE XXXIII

Figs. 1-4. *Fuchsia speciosa.*

Fig. 1. X-section of very young ovary (X 68.7). Same stage as Plate V, Fig. 10.

Fig. 2. X-section (X 31.2) of older ovary.

Fig. 3. X-section of ovary of Fig. 4. (X 15.5).

Fig. 4. L-section of an older flower. (X 15.5).

Figs. 5-11. *Citrullus vulgaris.* Figs. 5-10 (X 68.7); Fig. 11 (X 31.2).

Fig. 5. Beginning of flower bud.

Fig. 6. Sepal lobes appearing.

Figs. 7, 8, 9, 10 and 11. L-sections of young flowers, showing the beginnings and development of sepals, petals, stamens and pistils.

Figs. 12-15. *Sanicula canadensis* (X 68.7).

Fig. 12. Beginning of flower bud, a;

Fig. 13. Three flower buds in different stages of development.

Fig. 14. Carpels have appeared.

Fig. 15. Two ovules growing up into central cavity.

PLATE XXXIV

Figs. 1 and 2. *Sanicula canadensis* (X 31.2). Longisections of flower, with inferior ovary and ovules.

Figs. 3-10. *Galium aparine.* Figs. 3-8 (X 68.7). Figs. 9 and 10 (X 31.2).

Fig. 3. Longisection of young flower. Receptacle flattened.

Fig. 4. L-section; sepals and petals appear.

Fig. 5. Same. Carpels and placenta forming.

Fig. 6. Placenta two-lobed.

Figs. 7 and 8. Further development of parts, with two ovules.

Fig. 9. X-section of ovary cut at "m" Fig. 8.

Fig. 10. Same cut below Fig. 9.

Figs. 11-17. *Sambucus canadensis.* (X 68.7).

Fig. 11. L-section of fl. bud; lobes of sepals.

Figs. 12 and 13. Beginning and growth of petals.

Fig. 14. Stamens appearing.

Fig. 15. Growth of parts shown in Fig. 14.

Fig. 16. L-section. Carpel lobes just beginning.

Fig. 17. Placenta growing out from central cavity.

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Plate XXXV

Figs. 1 and 2. *Sambucus canadensis* (X 31.2).

Fig. 1. Longisection of fl; beginning of ovules.

Fig. 2. Further development of parts already begun.

Figs. 3-9. *Valeriana officinalis* (X 68.7).

Fig. 3. Longisection of two fl. buds.

Fig. 4. Two fl. buds showing different stages of development.

Fig. 5. L-sections; petals present.

Figs. 6 and 7. L—sections; carpels appearing and developing.

Fig. 8. Elongation of two regions, forming inferior ovary and corolla tube.

Fig. 9. Inferior ovary and ovules.

Figs. 10-16. *Helianthus annuus.* (X 68.7).

Fig. 10. Flower buds appearing on flattened receptacle.

Fig. 11. One flower. Lobes of petals appearing.

Figs. 12 and 13. Beginning of stamens and carpels.

Figs. 14 and 15. Further development of floral organs.

Fig. 16. L-section of inferior ovary, stamens adnate to petals.

Plate XXXVI

Figs. 1-14. (X 68.7). L-sections of similar stages in the development of the floral leaves of the Monocotyledons and the Dicotyledons.

Figs. 1-6. Monocotyledons.

1. *Sisyrinchium angustifolium.*
2. *Gladiolus gandavensis.*
3. *Freesia refracta.*
4. *Iris germanica.*
5. *Canna indica.*
6. *Musa sapientum.*

Figs. 7-15. Dicotyledons.

7. *Malus ioensis.*
8. *Ribes aureum.*
9. *Fuchsia speciosa.*
10. *Citrullus vulgare.*
11. *Sanicula canadensis.*
12. *Galium aparine.*
13. *Sambucus canadensis.*
14. *Valeriana officinalis.*
15. *Helianthus annuus.*

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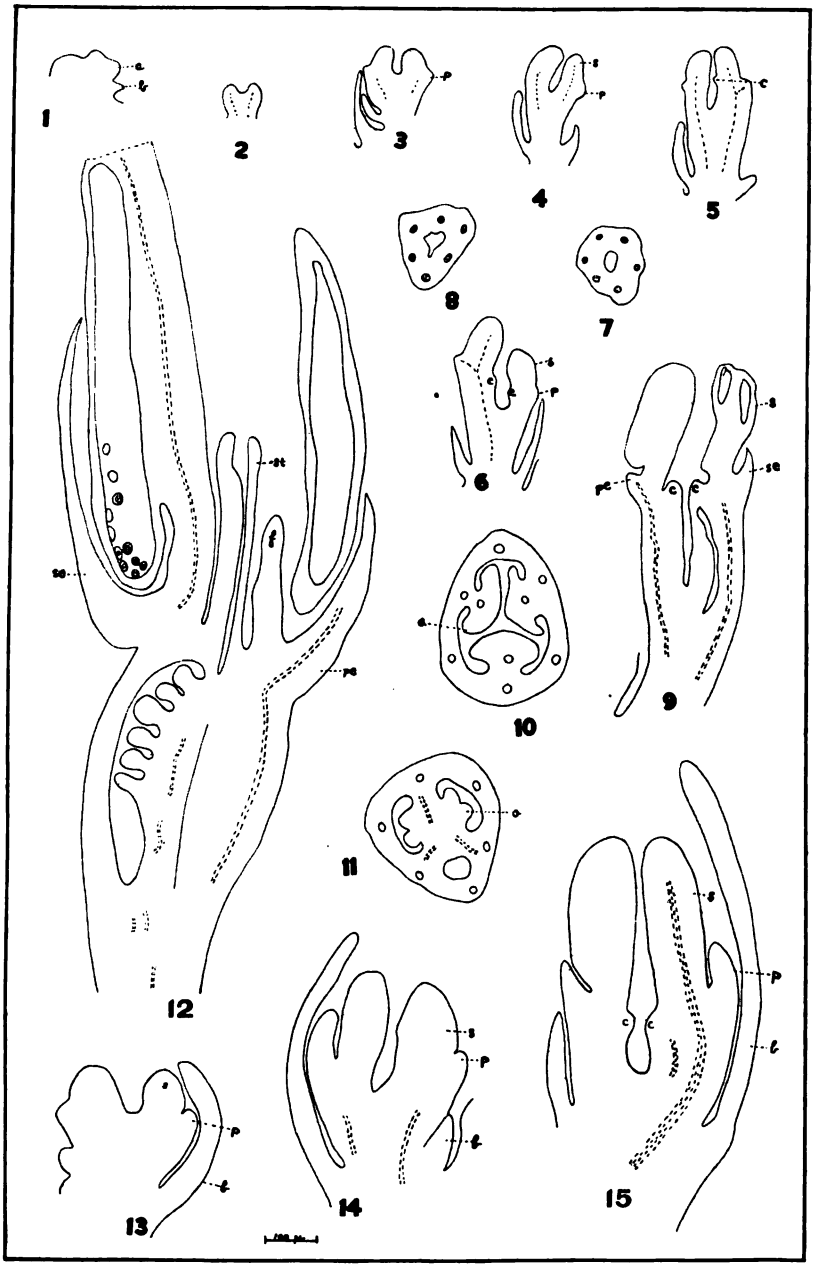

PLATE XXVIII

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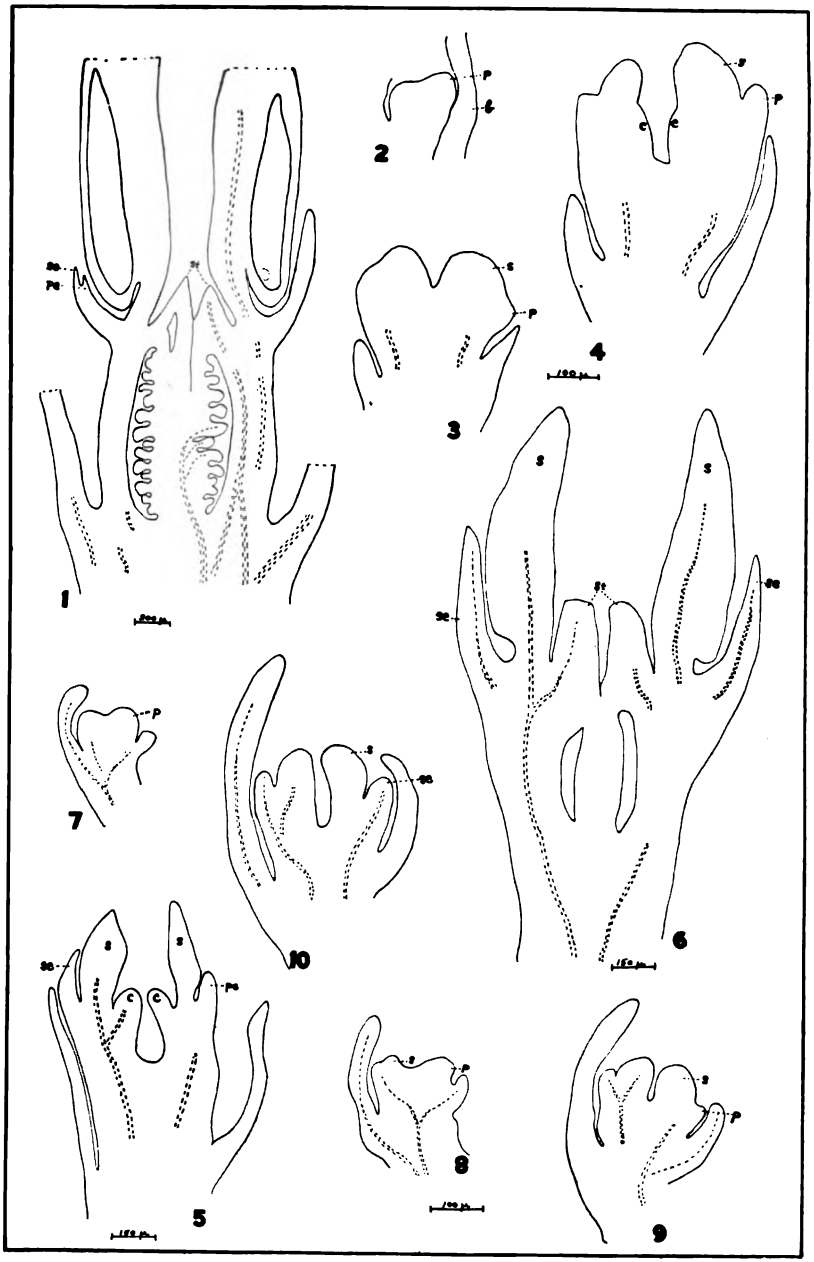

PLATE XXIX

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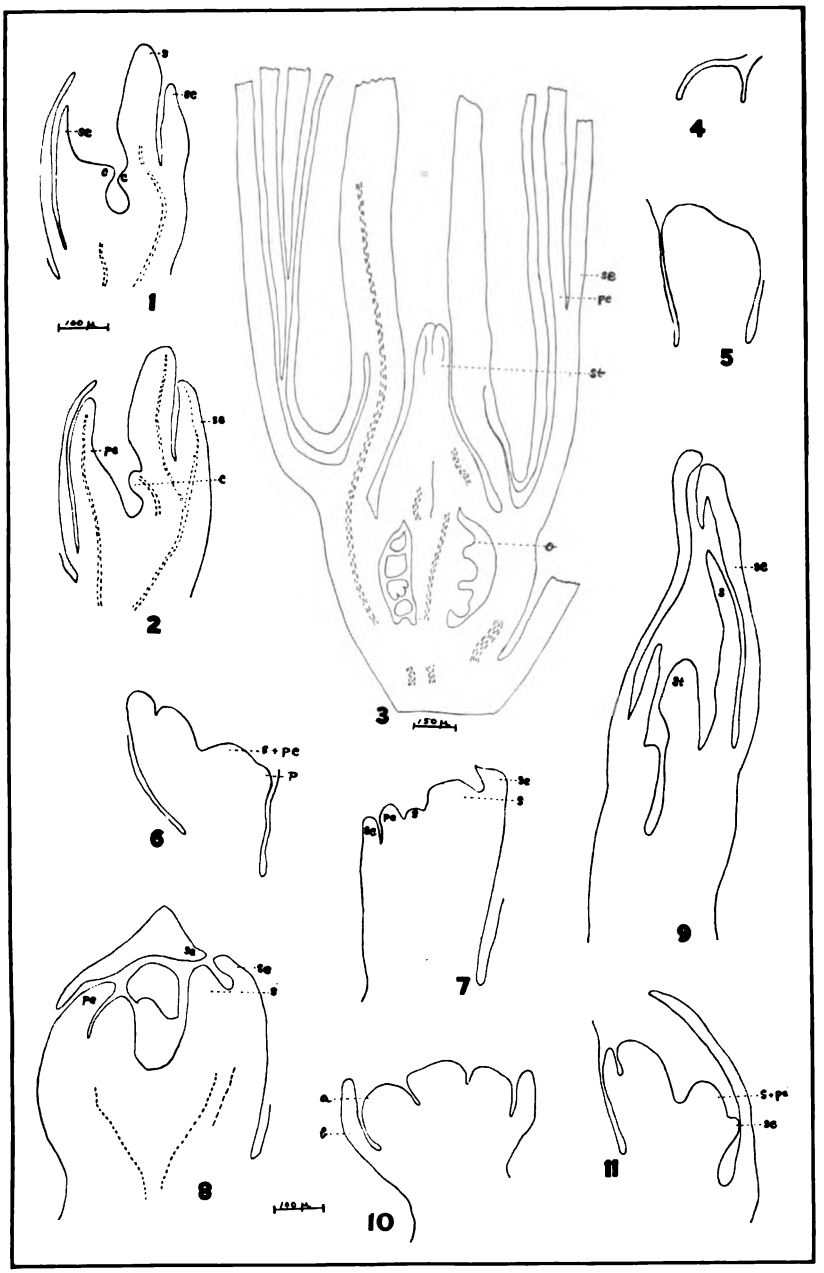

PLATE XXX

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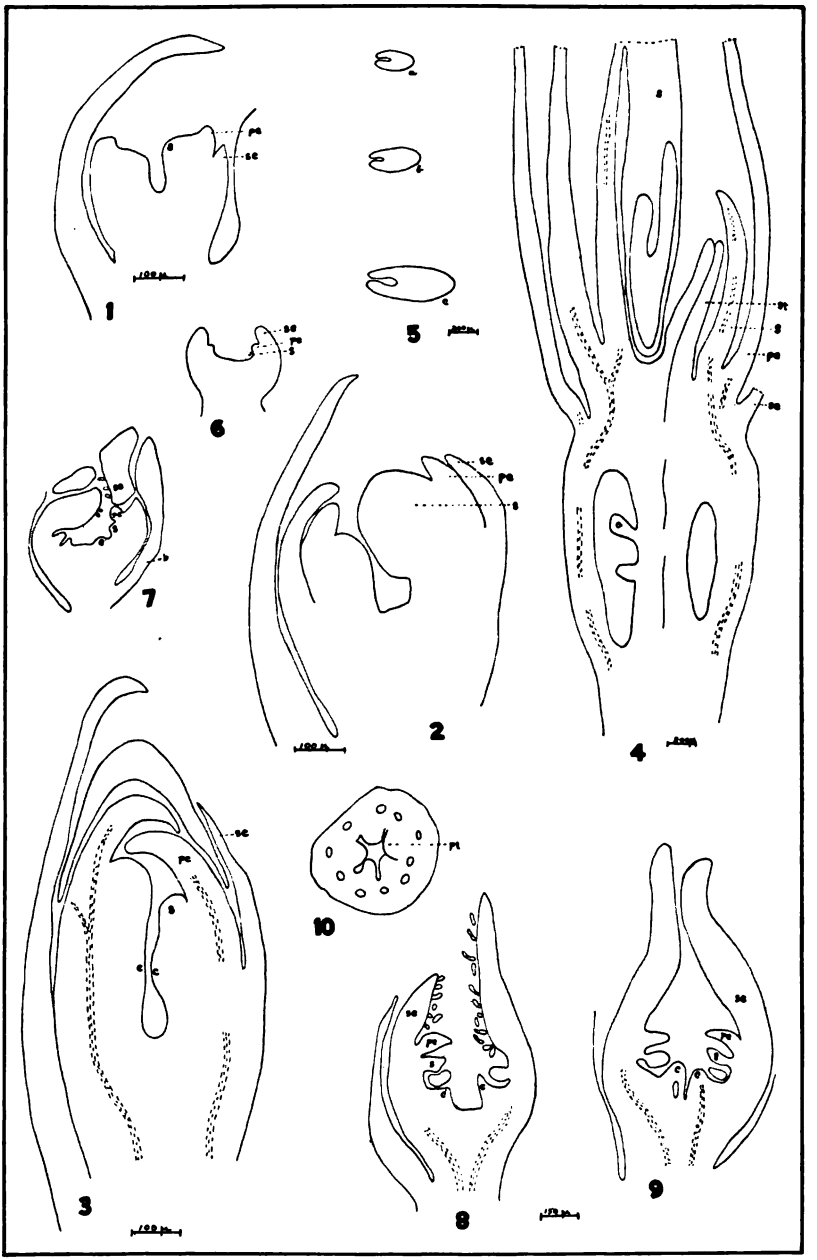

PLATE XXXI

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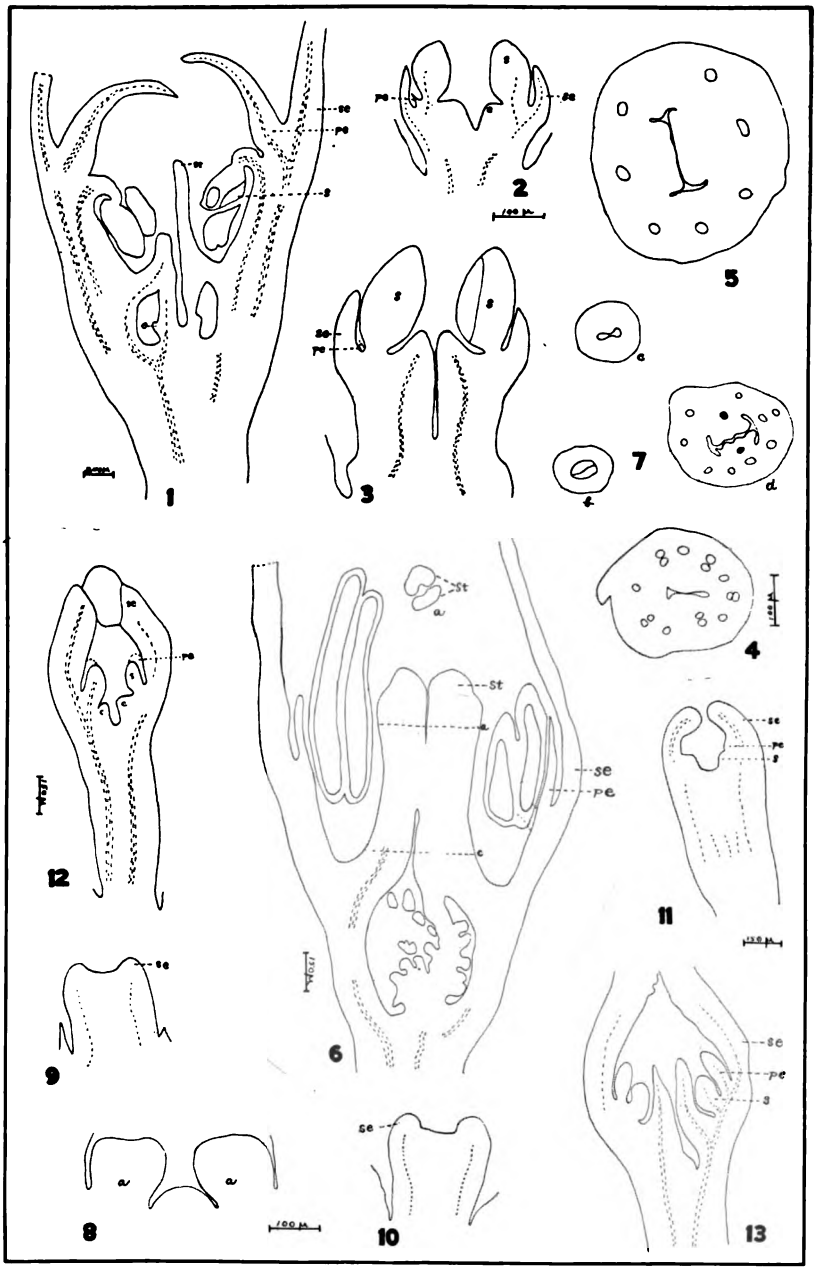

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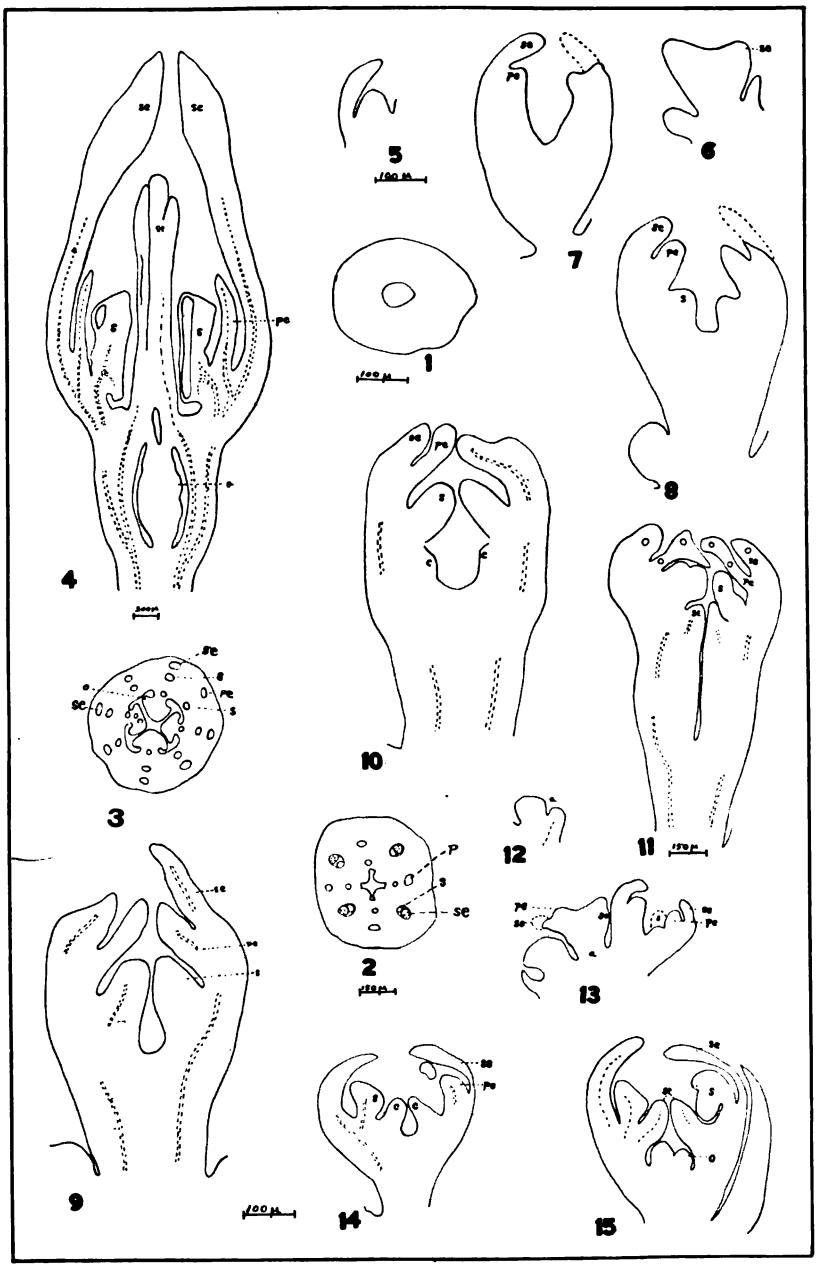

PLATE XXXIII

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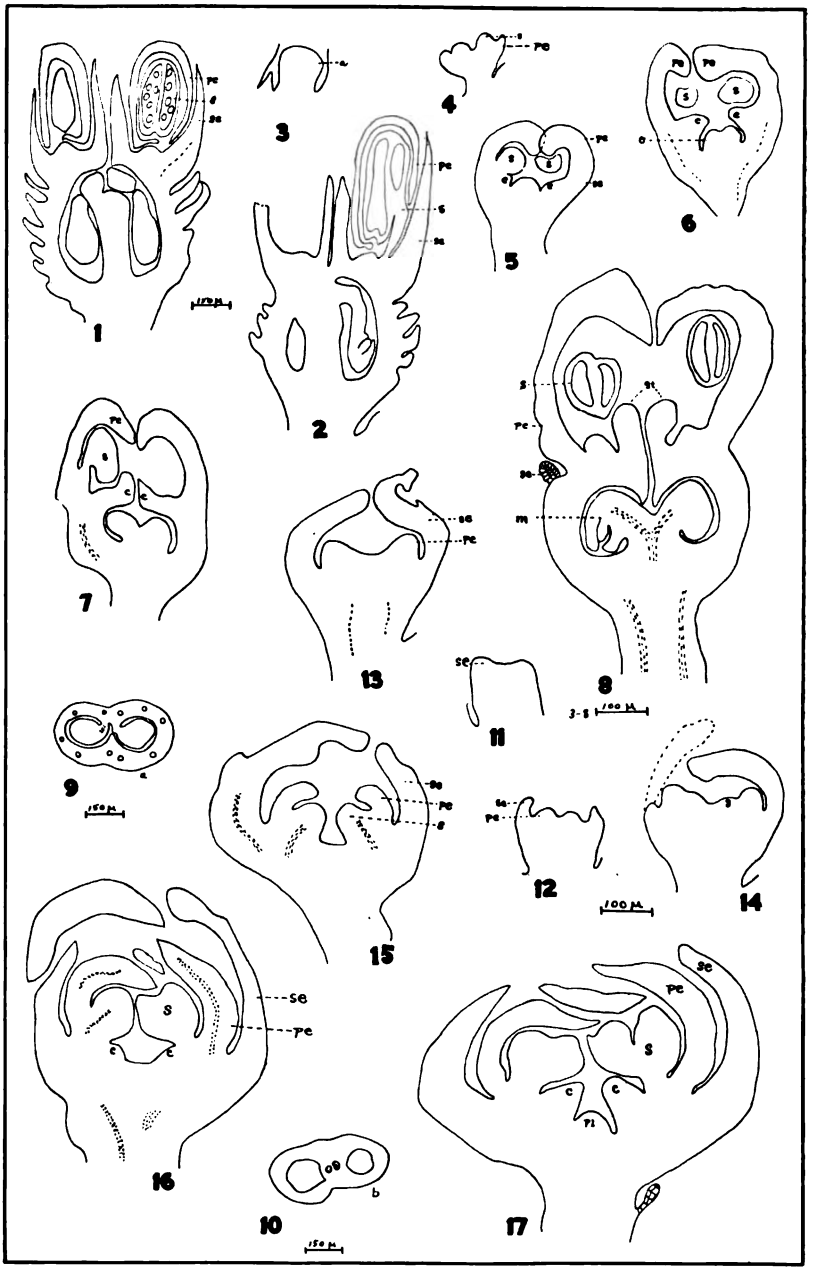

PLATE XXXIV

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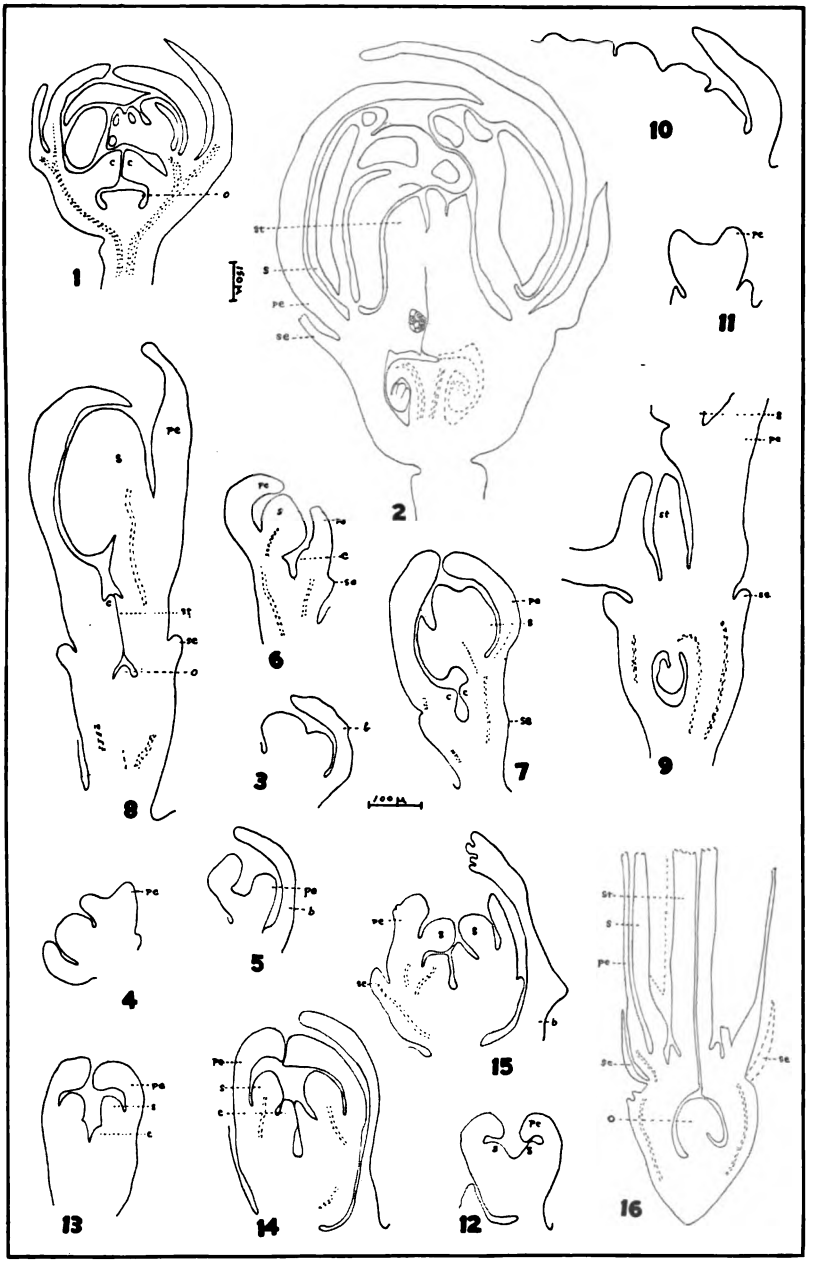

PLATE XXXV

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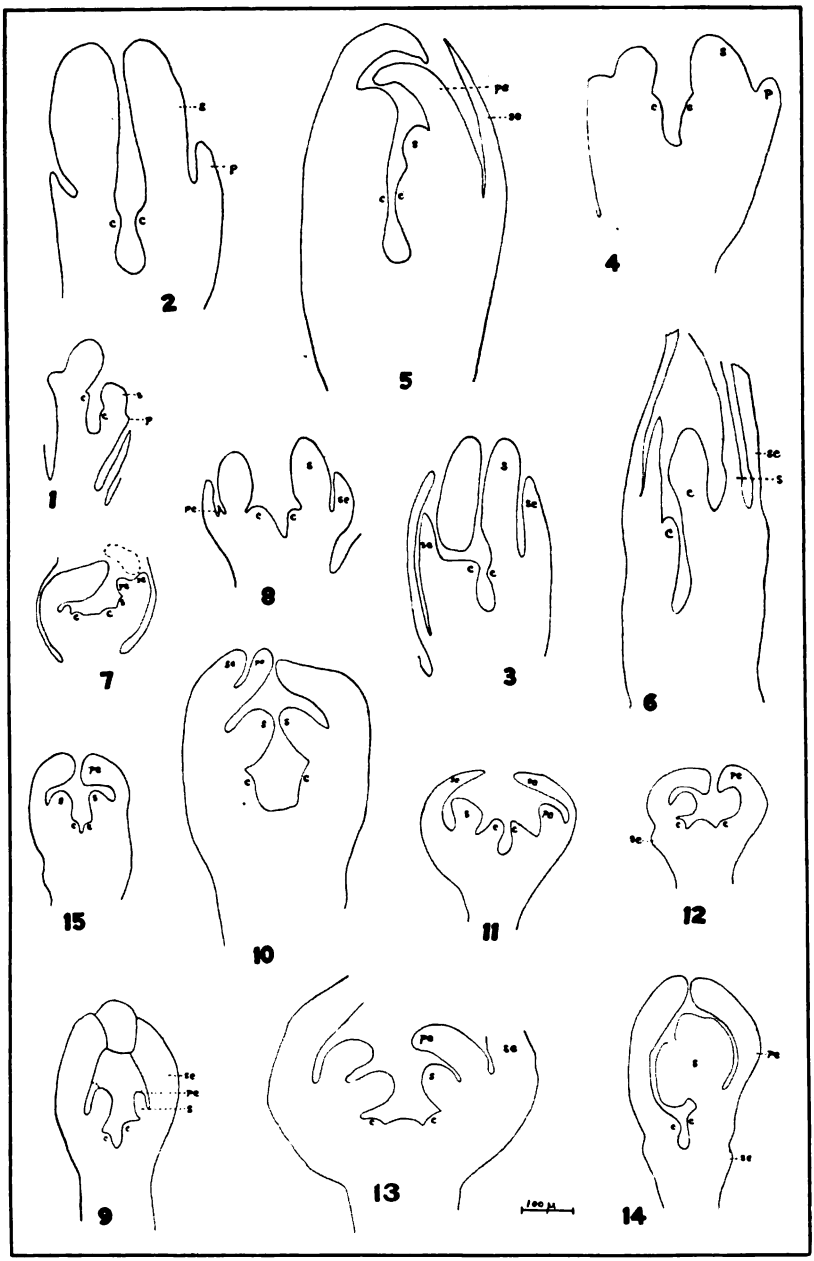

PLATE XXXVI

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ACANTHOCEPHALA OF THE GENERA CENTRORHYNCHUS AND MEDIORHYNCHUS (NEW GENUS) FROM NORTH AMERICAN BIRDS*

H. J. Van Cleave

Introduction

The writer has made a thorough study of the avian Acanthocephala in the collections of the U. S. Bureau of Animal Industry. In this study a number of specimens comprising four undescribed species have been discovered in which the proboscis receptacle finds its insertion near the middle of the proboscis wall. This method of insertion is characteristic of but a single known genus of Acanthocephala. The morphology of the four newly discovered species possessing a proboscis receptacle of this type varies too broadly in points of fundamental structure to permit of including all four species within the Genus Centrorhynchus. But one species of the four, and that represented by a single individual in the collection under consideration, agrees with the characters of the genus Centrorhynchus as given by Lühe (1911:41). For the other three species the writer has found it necessary to create a new genus, the characters of which are enumerated in another part of this paper.

Methods

The specimens for study have been stained in toto with Ehrlich's acid hematoxylin, dehydrated, cleared in synthetic oil of wintergreen, and mounted in damar. All the drawings have been made with a camera lucida.

Genus Centrorhynchus Lühe 1911

The genus Centrorhynchus comprises a well defined group of parasitic worms, belonging to the Class Acanthocephala, which reach maturity in the alimentary canal of birds. One of the most striking characteristics of this genus is the insertion of the proboscis

*Contributions from the Zoological Laboratory of the University of Illinois, No. 76.

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receptacle in the middle of the proboscis wall. This peculiar departure from the ordinary arrangement of the organs and parts of the body, while not the sole means of distinguishing the members of this genus, has lead to considerable confusion among various workers. The attempt on the part of some investigators to homologize the basal region of the proboscis with the neck characteristic of some of the other genera of Acanthocephala has been most distinctly refuted by Lühe (1912:274). He has shown that if the insertion of the proboscis receptacle marks the boundary between neck and proboscis the genus Gigantorhynchus must be considered as having no true proboscis for, according to Lühe's observations, the proboscis receptacle in the genus Gigantorhynchus finds its insertion at the tip of the organ of fixation, which, according to the proposed distinction, would of necessity be considered an armed neck. The folly of this argument is self evident. There is no reason for doubting that the organ of attachment in the genus Gigantorhynchus is a true proboscis. Similarly the spined regions both anterior to and posterior to the insertion of the proboscis receptacle constitute the proboscis of the Centrorhynchi.

Little has been done toward establishing the synonomy within the genus Centrorhynchus. Lühe (1911:41) has listed the forms from central Europe which might be attributed to it but has added the statement that at least some of the names included in his list are certainly synonyms. Kostylew (1914:186) has given a list of four species which he considered as valid for this genus but has not presented the data used in reaching his conclusion.

Of the species attributed to the genus Centrorhynchus but a single one has been reported from North America. Leidy (1888:22) has recorded the occurrence of individuals by him determined as "*Echinorhynchus caudatus* Zeder" from the swallow tail kite, *Elanoides forficatus* (*Elanoides furcatus*)* and two specimens of the same species from the owl *Scotiaptex nebulosa (Strix nebulosa)*. The specific identity of these specimens with the European species must be sharply questioned, for, as earlier works of the writer have shown, the Acanthocephala of North America, and

*Here, and elsewhere in the text, scientific names of birds quoted from another writer or taken from records accompanying the collections follow in parenthesis after the name given for the species in the A. O. U. Check List.

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especially those from fresh-water and terrestrial hosts, constitute a list of forms in the main peculiar to the American continent. A fuller account of the evidences of this development is given at the end of this paper.

On the basis of the present study it becomes impossible to determine whether the Acanthocephala described by Leidy belong to the Centrorhynchi or the genus Mediorhynchus. The only valid record of a species of Centrorhynchus from North America is in a single collection of one individual in the Collections of the Bureau of Animal Industry. A comparison of this specimen with descriptions of other species of the genus has revealed differences which make it necessary to consider this a new species. The specific diagnosis follows.

CENTRORHYNCHUS SPINOSUS NOV. SPEC.

(Figs. 1-3)

Specific diagnosis. With the characters of the genus. Proboscis closely set with numerous hooks. Species description based on a single female which becomes type. Body 20 mm. long; diameter anterior part of body slightly larger (6.6 mm.) than posterior part (0.5 mm.); posterior extremity slightly pointed, conical. Proboscis 0.65 mm. long; with hooks of two distinct types, those anterior to insertion of proboscis receptacle with recurved roots; strongest hooks (0.038 mm. long) appearing near the middle of the proboscis just behind the cylindrical apical portion. Hooks in thirty-two longitudinal rows of about twenty-four hooks each. Hooks posterior to insertion of proboscis receptacle thornlike, about 0.05 mm. long, often with recurved tips. Proboscis constricted at insertion of proboscis receptacle, diameter at constriction 0.25 mm.; posterior portion (0.35 mm. in diameter) not sharply set off from body proper; slightly swollen anterior to insertion of receptacle; tip smaller, cylindrical, 0.17 mm. in diameter.

Type host *Herodias egretta (Ardetta egretta),* in intestine. Collected by Hassall, Sept. 1894. Type deposited in the U. S. Bureau of Animal Industry Helminthological Collections. Catalog number Hassall Collection 6307.

In general body form this species closely resembles the figures given by Lühe (1911 Fig. 54) for *C. aluconis.* Unfortunately the

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male of *C. spinosus* is unknown so a comparison of the male organs with those of *C. aluconis* must be deferred until other materials are available. One of the most strikingly characteristic points about this species is the abundance of the spines (see Figs. 1 and 2). The entire proboscis, even under a low power of the microscope, fairly bristles with projecting spines. While the hooks of the anterior part of the proboscis are stronger than those posterior to the insertion of the proboscis receptacle (see Fig. 3) the difference in size is not as conspicuous as in the species of Mediorhynchus described in this paper.

Mediorhynchus new genus

The remaining three species differ from the characterization of the genus Centrorhynchus in the following particulars: (1) The males possess eight rounded or pear shaped cement glands instead of the three long tubular cement glands described for Centrorhynchus. (2) The wall of the proboscis receptacle is composed of a single muscular layer instead of two layers as specified by Lühe for Centrorhynchus and shown in figure 2 of *C. spinosus*. (3) The invertors of the proboscis pass through the sides of the proboscis receptacle considerable distance from its base and continue backward through the body cavity as the retractors of the proboscis receptacle, while in Centrorhynchus the invertors pass through the wall of the receptacle at its rounded posterior extremity (see Fig. 10).

Generic diagnosis. Acanthocephala of medium size reaching sexual maturity in the alimentary canal of birds. Proboscis receptacle inserted near the middle of the proboscis wall. Receptacle a single walled muscular sac with invertors of proboscis passing through its wall some distance anterior to the posterior tip of the receptacle. Central nervous system near the center of the proboscis receptacle between the invertor muscles. Cement glands of male a compact mass of rounded or pear shaped glands, usually eight in number. Proboscis hooks of two distinct types; those anterior to the insertion of the proboscis receptacle in surface view with flask shaped roots, bases of roots broad; those on the posterior portion of the proboscis without reflexed roots. Embryos with three concentric membranes.

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Descriptions of three species belonging to the genus Mediorhynchus follow:

MEDIORHYNCHUS PAPILLOSUS NOV. SPEC.

(Figs. 4-10)

Specific diagnosis. With the characters of the genus. Proboscis armed with inconspicuous hooks, each hook embedded in a papilla (see Figs. 5 and 8.). Body of both sexes cylindrical, almost uniform diameter throughout. Type male 9.3 mm. long; diameter 0.75 mm., tapering to 0.57 mm. at base of proboscis, and tapering slightly at posterior extremity. Cement glands (see Fig. 7) eight, rounded to pyriform, in compact mass slightly posteriad of posterior testis. Testes two, elongated, elliptical, contiguous; in chief axis of body. Proboscis 0.65 mm. long; largest diameter 0.30 mm., near the middle. Lemnisci 3 mm. long.

Type female 18 mm. long, diameter 0.75 mm., tapering to 0.57 mm. at base of proboscis. Embryos 0.038 to 0.047 mm. long by 0.018 to 0.024 mm. across, with three concentric membranes (see Fig. 9.).

Proboscis anterior to insertion of proboscis receptacle with eighteen longitudinal rows of six or seven hooks each, longest hooks 0.027 mm. long, each hook with a root process (0.040 mm. long) usually longer than the recurved spine (see Fig. 6.). Surface view of roots pyriform (see Fig. 8.). Hooks posterior to insertion of proboscis receptacle without reflexed roots; thornlike, with tips bent posteriorly almost at right angles to axis of the spine; four to six hooks in a longitudinal row.

Host *Myiochanes virens (Contopus virens)*. Collected May 30, 1892, by Albert Hassall. Type male and type female deposited in the U. S. Bureau of Animal Industry Helminthological Collection. Catalog number 6320 Hassall Collection.

Besides the types of *M. papillosus* the collections of the U. S. Bureau of Animal Industry contain one specimen of this species, a male from the intestine of *Porzana carolina* in the Hassall collection, catalog number 6303. The locality from which this individual was taken is not given in the records accompanying the specimen.

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MEDIORHYNCHUS GRANDIS NOV. SPEC.

(Figs. 11-14)

Specific diagnosis. With the characters of the genus. Specific description based upon the study of one male and three fully mature females, constituting two collections from different hosts. Body of females 27 to 35 mm. long, practically cylindrical; diameter anterior region 1.2 mm.; maximum diameter considerable portion in middle of body 0.9 to 1.4 mm.; diameter near posterior extremity 0.7 to 1.1 mm. Anterior and posterior extremities of body flexed ventrally. Anterior part of body just behind proboscis expanded and sharply set off from neck. Proboscis of all specimens partially inverted; largest female anterior to insertion of proboscis receptacle approximately 0.6 mm. long if extended (all but 0.3 mm. inverted in type); proboscis posterior to insertion of receptacle 0.6 mm. long. Hooks in anterior region of proboscis with massive roots; twelve longitudinal rows of approximately four hooks each (two in each row show on surface of inverted proboscis). Basal portion of proboscis with numerous small spines, not in perfect rows but about thirty longitudinal rows with three to six spines each. Hooks on anterior proboscis 0.05 mm. long (measurement taken as longest straight line from the tip of the spine to the region where the hook and root join). Length of root 0.075 to 0.086 mm. (from base of root to top of angle between root and hook). Embryos with three concentric membranes, about twice as long as broad; 0.043 by 0.021 mm.

Male 8.2 mm. long. Maximum diameter 1 mm; diameter posterior tip 0.18 mm.; anterior to tip 0.5 mm. Diameter anterior end of the body proper 0.61 mm. Lemnisci about 2 mm. long. Testes oval, slightly separated, 1.2 mm. long and 0.35 mm. wide. Eight cement glands, each rounded, usually pear shaped.

Type host *Quiscalus quiscula,* in intestine. Cotypes deposited in U. S. Bureau of Animal Industry Helminthological Collection. Catalog number Hassall Collection 6319.

One fully mature female determined by the writer as belonging to this species has been found in the intestine of *Sturnella magna.* Collected by C. S. Brimley, Nov. 30, 1902, in North Caro-

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lina. In the U. S. Bureau of Animal Industry Parasitological Collection. Catalog number 6772.

MEDIORHYNCHUS ROBUSTUS NOV. SPEC.

(Figs. 15 and 16)

Specific diagnosis. With the characters of the genus. Body both sexes robust, medium length; largest diameter near the middle, tapering slightly toward either end; dorsal surface slightly convex. Proboscis small, setting on obliquely truncated anterior end of body, pointing slightly ventrad. Lacunar system of subcuticula highly developed. Lemnisci about one-fourth to one-third the length of body. Specific descriptions based on one male and one female which become types.

Type female 16 mm. long. Maximum diameter body proper 2.4 mm., diameter posterior extremity 0.8 mm., anterior extremity 0.9 mm. Proboscis short, globular, 0.2 mm. in diameter; tip partially inverted and base partially retracted within body; exposed portion anterior to insertion of proboscis receptacle armed with twenty-four longitudinal rows of hooks. Hooks very small and inconspicuous with small pyriform roots 0.032 mm. long. Each hook in an elevation of the proboscis wall but not in a distinct papilla. Basal region of proboscis entirely retracted within body, hooks not observable.

Type male 7 mm. long, maximum diameter 1.25 mm.; diameter posterior extremity 0.52 mm,; anterior extremity the same. Proboscis short, globular, 0.156 mm. in diameter, partially inverted. Hooks small, inconspicuous, about 0.025 mm. long; only about 0.005 mm. extending beyond the elevations on proboscis.

Embryos 0.038 mm. long by 0.016 mm. wide.

Type host *Icteria virens* in intestine. Collected by A. Hassall at Washington, D. C., in June 1893. Type male and female deposited in the Helminthological Collection of the U. S. Bureau of Animal Industry, catalog number 2316.

FAMILY CENTRORHYNCHIDÆ

When Hamann (1892:195-197) first pointed out the presence within the Acanthocephala of distinct groups and showed the necessity of recognizing a number of genera to displace the old all

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inclusive genus Echinorhynchus he founded three families; the Echinorhynchidæ, the Gigantorhynchidæ, and the Neorhynchidæ. The latter by recent change in name of the type genus becomes Neoechinorhynchidæ. Within each of these families he recognized a single genus. Since his work on this subject a long list of Acanthocephalan genera has been created but usually with the creation of a new genus the founder has neglected to point out the affinities of the genus. As a result today there are few references to groupings of genera into larger groups or families. As a start toward organizing this part of the system the writer proposes that since the genera Centrorhynchus and Mediorhynchus have the same type of proboscis receptacle and both reach sexual maturity in the alimentary canal of birds, they should be united to form a family which should take the name Centrorhynchidæ from the name of the oldest genus.

Family Centrorhynchidæ. Diagnosis. With the characters of the Class Acanthocephala. Living as mature adults in the alimentary canal of birds. Proboscis receptacle inserted near the middle of the proboscis wall.

The American Acanthocephala as a Distinctive Fauna

The development of a typical American fauna among the Acanthocephala, independant from and in time of relatively remote separation from the European fauna, is evidenced in the extent to which the American representatives of a genus differ from the European representatives upon which by far the greater amount of work has been done. In many cases a generic diagnosis based entirely upon European representatives of a genus fails in some points of detail to permit of the inclusion of American species subsequently discovered. In the genus Neoechinorhynchus the two species *N. gracilisentis* (Van C.) and *N. longirostris* (Van C.) show structures and hook arrangement at considerable variance from the conditions typical of described European forms. On the other hand the three species of the genus, *N. emydis* (Leidy), *N. cylindratus* (Van C.) and *N. tenellus* (Van C.), closest in their affinities to the European species agree among themselves in possessing eight syncitial cells in the cement gland. Bieler (1913:235) has called attention to the fact that the two generally recognized Euro-

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pean species *N. rutili* (Müll.), the common fresh-water species of Europe, has twelve nuclei in the cement gland, while *N. agilis* (Rud.), the marine representative of the genus, possesses eight nuclei in the cement gland of the male. These with other differences in structure of European and American Neoechinorhynchi would tend to indicate that the fresh-water representatives of the genus have either had (a) an independant origin on the two continents or (b) each developing from marine species of the genus at one time common to the two continents they have been separated a sufficient length of time to allow distinctive characteristics to develop in the two groups. Of these possibilities the latter seems the more plausible.

In the genus Filicollis the writer (1916:132) found it necessary to suggest a modification of the original characterization of the genus in order that *F. botulus* Van C. be admitted to a position near to *F. anatis* (Schrank) which general body structure and hosts of the two parasites demanded. Similarly in the genus Arhythmorhynchus the writer (1916a:169) has described two North American species which, though agreeing with the European species in fundamental generic characteristics, differ from them in some points of detail of structure.

Finally, in the Centrorhynchidæ the discovery of a new genus from North America adds still further evidence of the trend in North America toward the development of a distinctive Acanthocephalan fauna.

Summary

A study of avian Acanthocephala in the Collections of the U. S. Bureau of Animal Industry has revealed four new species, one belonging to the genus Centrorhynchus, and three with characters such as to prevent inclusion in any known genus. For the latter a new genus, Mediorhynchus, has been created.

The two genera Centrorhynchus and Mediorhynchus agree in the method of insertion of proboscis receptacle, and in the fact that both occur as parasites in the alimentary canal of birds. They differ in the size, shape, and number of the cement glands of the

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male; in the structure of the wall of the proboscis receptacle; and in the relations of the invertors of the proboscis to the proboscis receptacle.

Upon the basis of agreement the writer has suggested the establishing of a new family Centrorhynchidæ to include these two genera.

Evidence has been assembled to show the tendency toward the development of an Acanthocephalan fauna peculiar to terrestrial and fresh-water hosts of the North American Continent.

A key to the species of Centrorhynchidæ from North American birds is given.

KEY TO THE SPECIES OF THE FAMILY CENTRORHYNCHIDÆ FROM NORTH AMERICAN BIRDS

Acanthocephala parasitic in birds, with the proboscis receptacle inserted near the middle of the proboscis wall.—Family Centrorhynchidæ.

1. (2) Proboscis receptacle a two layered muscular sac, cylindrical, with the invertors of the proboscis passing through the posterior rounded tip and continuing backward through the body cavity as the retractors of the proboscis receptacle.—Genus Centrorhynchus. A single species, *C. spinosus*, reported from North America.

2. (1) Proboscis receptacle a single layered muscular sac with the retractors of the proboscis receptacle passing from its sides some distance anterior to the posterior tip. Receptacle not cylindrical in form. Genus Mediorhynchus 3

3. (6) Anterior and posterior regions of proboscis with the same number of longitudinal rows of hooks.....4

4. (5) Twenty-four longitudinal rows of hooks on proboscis. Maximum diameter of body : length of body :: 1 : 5 (or 6). .....*M. robustus*

5. (4) Eighteen longitudinal rows of hooks on proboscis. Maximum diameter of body : length of body :: 1 : 9....*M. papillosus*

6. (3) Twelve longitudinal rows of hooks on anterior region of proboscis; thirty on posterior region.....*M. grandis*

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EXPLANATION OF PLATES

Abbreviations used:

b. brain
c. g. cement gland
e. egg mass
ins. point of insertion of proboscis receptacle on proboscis wall
i. p. invertor of proboscis
l. lemnisci
p. r. proboscis receptacle
r. p. retractor of proboscis receptacle
t. a. anterior testis
t. p. posterior testis

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PLATE XXXVII

Centrorhynchus spinosus nov. spec.

Fig. 1. Type female showing body form and general arrangement of parts.

Fig. 2. Proboscis and anterior region of body, showing also insertion of proboscis receptacle and location of the retractors of the receptacle with reference to the wall.

Fig. 3. Profile of the same proboscis showing a single longitudinal row of hooks.

PLATE XXXVIII

Mediorhynchus papillosus nov. gen. et nov. spec.

Fig. 4. Entire male from intestine of *Prosana carolina* showing arrangement of organs.

Fig. 5. Proboscis and anterior region of body, surface view, of type male from *Myiochanes virens*.

Fig. 6. Profile of proboscis shown in figure 5, showing a single longitudinal row of hooks.

Fig. 7. Posterior end of body of male in optical section showing especially the shape, number, and arrangement of the cement glands characteristic for the genus Mediorhynchus.

Fig. 8. Surface view of roots and papillæ from proboscis.

Fig. 9. Embryos from body of mature female.

PLATE XXXIX

Fig. 10. *M. papillosus*. Optical section of proboscis and anterior region of body showing attachment and structure of the proboscis receptacle and course taken by invertors of the proboscis through the wall of the receptacle.

Figs. 11, 12, 13, and 14 of *Mediorhynchus grandis* nov. spec.

Fig. 11. Anterior region of body of female.

Fig. 12. Proboscis of same specimen. In surface view the delicate spines on the posterior region of the proboscis are discernable only as small circular markings.

Fig. 13. Profile of proboscis showing single longitudinal row of hooks.

Fig. 14. Embryos from body cavity of female.

Figs. 15 and 16. *Mediorhynchus robustus* nov. spec.

Fig. 15. Male in optical section showing arrangement of organs.

Fig. 16. Embryos from body cavity of female.

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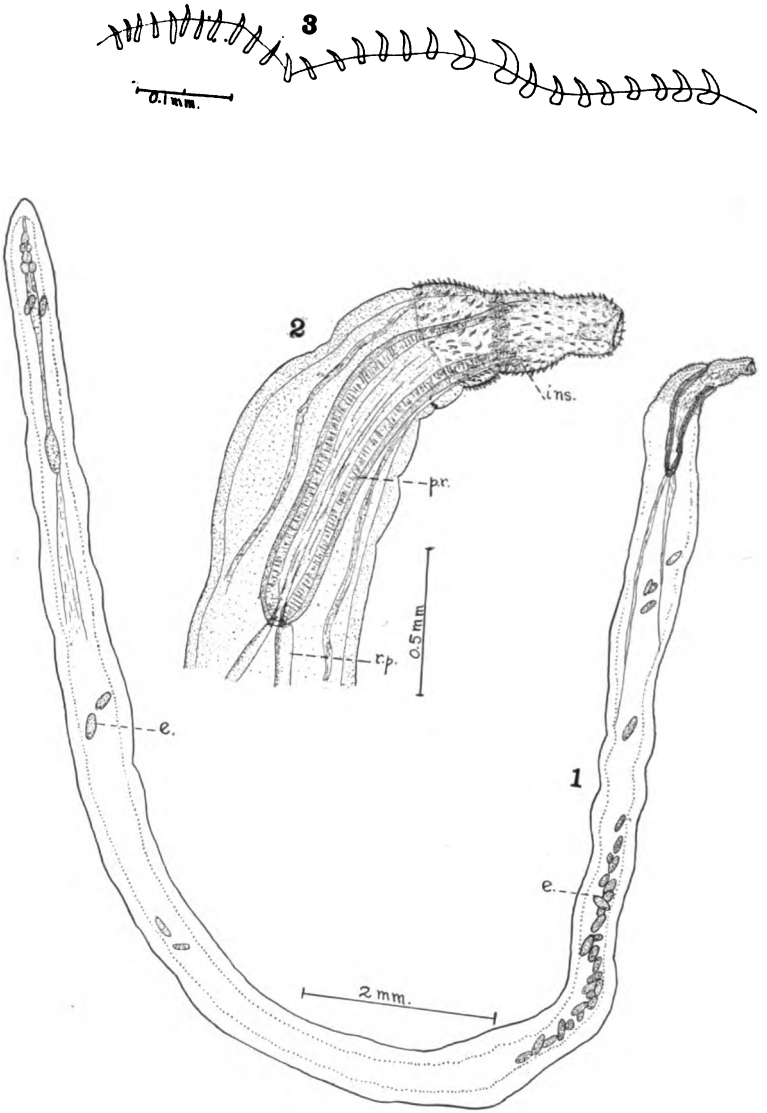

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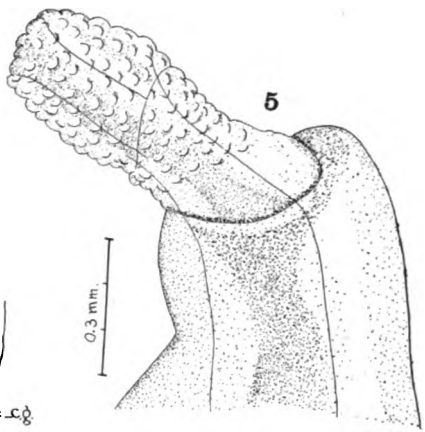

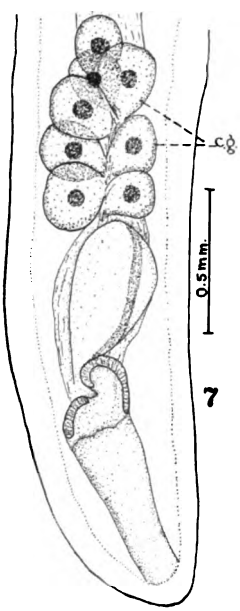

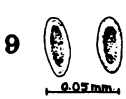

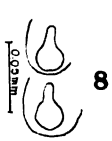

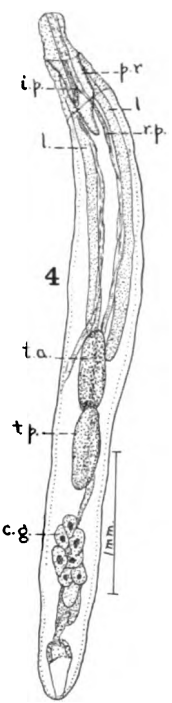

PLATE XXXVIII

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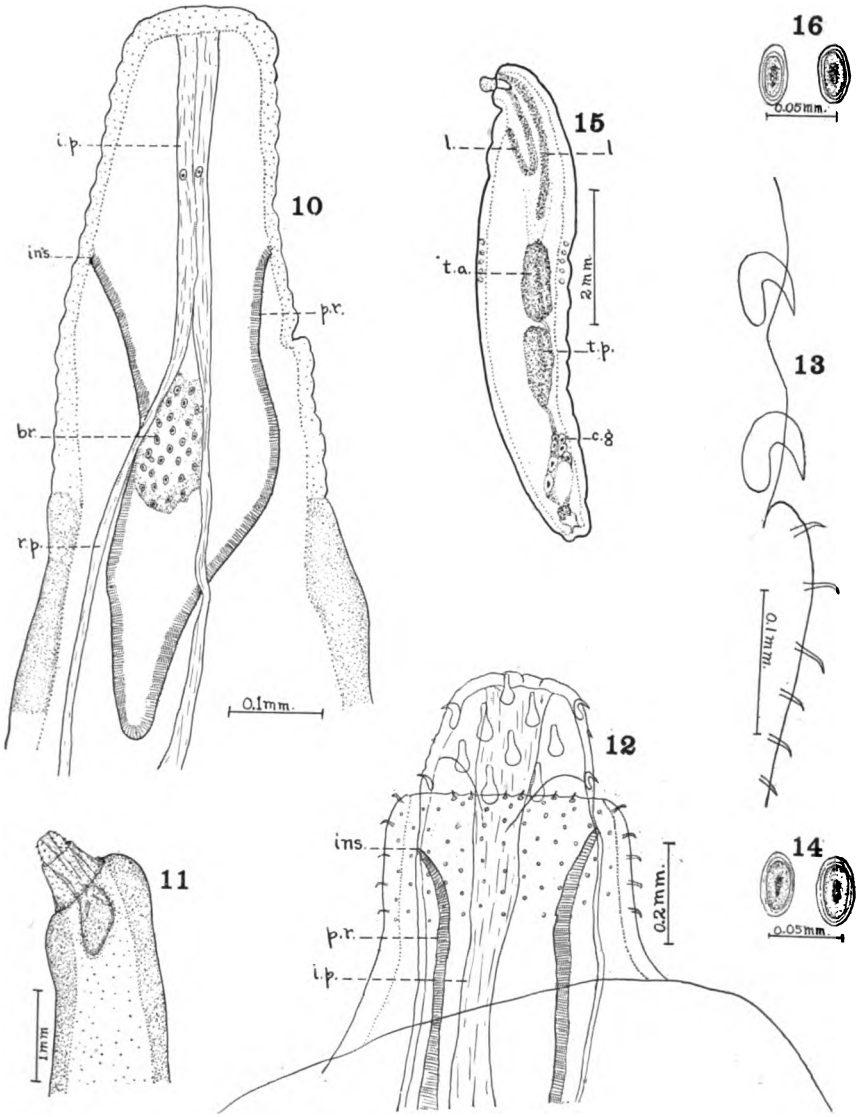

PLATE XXXIX

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THE GENUS ASPIDISCA EHRENBERG

HAROLD H. PLOUGH

The following systematic account of the genus Aspidisca was undertaken with the idea that at some future time the writer would use one of the species for a series of culture experiments. In order to do this intelligently an extensive study of the literature of the genus was suggested by Prof. G. N. Calkins. This study had disclosed one or two rather obvious mistakes in the recent literature, and showed the need of a new systematic account of the genus. The confusion in a relatively simple and homogeneous group like the Aspidiscas indicates that considerable work still remains to be done in systematizing the whole group of Hypotrichs.

ASPIDISCA EHRENBERG 1830

O. F. Muller 1773, 1780, 1786, 1788. Bory 1824. Ehrenberg 1830, 1831, 1833, 1838. Dujardin 1841. Perty 1852. Claparede at Lachmann 1857. Stein 1859. Fresenius 1865. Diesing 1865-6. Quennerstedt 1867-8. Fromental 1874. Mereskowsky 1878. Kent 1881. Rees 1884. Fabre-Domergue 1885. Perejaslawzewa 1886. Gourret et Roesser 1886, 1888. Calkins 1902. Hamburger und Buddenbrock 1911.

The genus Aspidisca was originally separated off from O. F. Muller's Trichoda by Ehrenberg 1830 and placed in a separate family Aspidiscina. The family was given the following rather fanciful characterization: "animaux polygastriques à carapace, ayant un canal intestinal distinct à double orifice, dont seulement celui de l'anus est terminal." Under Bory de St. Vincent's name Coccudina Dujardin 1841 and afterward Perty described several species which are probably Aspidiscas, though the fact can be established only in the case of Dujardin's Coccudina costata. The principle character which distinguishes the genus Coccudina from his other Ploesconien was the absence of a mouth. This does not stand for as Claparede et Lachmann 1857 first showed the mouth is at the base of a peristomial field of cilia on the left side and between the upper and lower plates of the cuirass. These investigators dis-

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tinguished Aspidisca from the other genera in their family Oxytrichina by the absence of frontal cirri. Stein 1859 again made of the genus a separate family under Ehrenberg's name and considered that it represented a connecting link between his family Chlamydodonta (Chilodon, Ergvilia etc.) and the Euplotina. Kent 1881 recognized that the main point of difference between this genus and the remainder of his family Euplotidae was "in the more rearward location of the peristome field and consequent non-projection of the adoral fringe of cilia beyond the lateral border," and included the genus in that family. This classification is generally accepted. The location of the peristome field as above described and the absence of marginal cirri are sufficient to distinguish the genus from the other genera of the family.

Description of the Genus Aspidisca Ehr. 1830.

Synonomy: Trichoda p p—Muller 1773, 1780, 1786, 1788.
Tribulina? Ratulus? p p—Bory 1824.
Oxytricha? p p Euplotes p p Loxodes plicatus ??—Ehrenberg 1838.
Coccudina p p—Dujardin 1841, and Perty 1852.
Onychaspis—Diesing 1856.
Monostylus ??—Perejaslawzewa.

Animalcules ovate, small, rigid, encuirassed, with a convex dorsal and a plain ventral surface; left side nearly straight, right sharply convex; the right border having a thickened margin; peristome limited to the left ventral side where it forms a small depression which may or may not just reach—but in no case extends beyond—the anterior border; associated with it is a simple fringe of adoral cilia which do not extend beyond the border; the ventral surface on the left side extends to a greater or less degree toward the right under the peristome, so that the latter is located in a pocket between the upper and lower surface; several large claw-like styles toward the anterior end and in the center of the ventral region; a variable number of posterior or anal styles arranged in a single row just inside the posterior ventral margin; anal aperture placed far back, debouching a little in advance of the anal styles. Cosmopolitan, fresh and salt water.

The number and position of the ventral and anal cirri has thus far been considered the important diagnostic character of the species of Aspidisca. Yet A. polystyla Stein was described as having from ten to twelve anal cirri, and a variety described by Calkins 1902 from Woods Hole has thirteen anal and eight rather than seven ventral cirri—which number had not previously been

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exceeded in the genus. Calkin's variety of A. hexeris Quenn. has also eight ventral cirri, while A. Andrewii Meresch.=A. hexeris has the seven very differently arranged. Fresenius 1865 described A. leptaspis with five anal styles, but Rees 1884 showed that one of these often frays out into three, making seven in all. Fabre-Domergue 1885 described this seven styled form as a new species A. crenata. It appears then that the number and position of the cirri is to a large degree variable, and that they must be discarded as of importance in differentiating species. A re-examination of the genus discloses that the species can be readily distinguished by the character of the stiff cuirass. This may be smooth or serrated dorsally, and the left border may be plain or incised so as to produce a varying number of posteriorly directed spurs. The form of the stiff cuirass is apparently extremely constant within the genus, and a few minutes observation of it will generally suffice to identify the species. In view of this fact it appears that A. lynceus and A. polystyla are really very similar, and further work may disclose that they are one and the same species. The latter of these species was placed in a separate subgenus-Onychaspis—by Stein and a separate genus by Diesing on the basis of its larger number of styles, yet one might easily be derived from the other by the continuation of the fraying out process which has been noted above in A. leptaspis. From the simple smooth bordered forms like A. lynceus it is possible that the spurred and serrated forms have been derived. On the basis of the form of the cuirass a simple key for the quick identification of the species of Aspidisca may be constructed.

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KEY FOR THE IDENTIFICATION OF THE SPECIES OF ASPIDISCA

A. Right and left border smooth.....a

B. Left border incised to form a single backwardly directed spur in the posterior third.....A. hexeris

C. Left border with two spurs, one in the anterior and one in the posterior third.....c

D. Left border with three spurs.....A. sedigita

a. Dorsal surface with recurved thorn-like appendage..*A. turrita
Dorsal surface without thorn.....a'

c. Dorsal surface and posterior border serrated.....A. leptaspis

Dorsal surface smooth.....A. lyncaster

a'. Ventral plate projecting beyond left border of carapace .....*A. costata
Ventral plate not projecting.....a"

a". Peristome reaching anterior border, anal cirri 5.....A. lynceus

Peristomial cilia not reaching anterior border, anal cirri more than 5.....A. polystyla

A. Right and left border smooth.

A. lynceus (Muller).

Trichoda lynceus Muller 1773, 1780, 1786, 1788.
Ratulus lynceus ? Bory 1824.
Aspidisca lynceus Ehrenberg 1838.
Coccudina crassa ? Dujardin 1841.
Aspidisca lynceus Claparede et Lachmann 1867 t6.

Body ovate, widest and somewhat truncate posteriorly; the marginal border of the carapace entirely even, the left one straight, the right one somewhat convex; the dorsal surface smooth, or marked longitudinally with three feeble furrows; the inferior surface bearing 7 ventral styles in two rows—anterior with 4, posterior with 3- 5 anal styles. L—1/540". Salt water.

Distribution: Baltic (Ostsee) at Hapsal (Eichwald), Warberg and Wisby (Quenn.), Wismar (Ehr. and Stein), Ostershelde (Rees), Mittelmeer Cette (Duj.), Gulf of Naples (Geta-Entz), Gulf of Mexico (Smith), Siberia and Egypt (Schewiakoff).

*Indicates the only forms so far reported in fresh water.

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This species is the type of the genus as correctly designated by Claparede et Lachmann, and later by Fromentel.

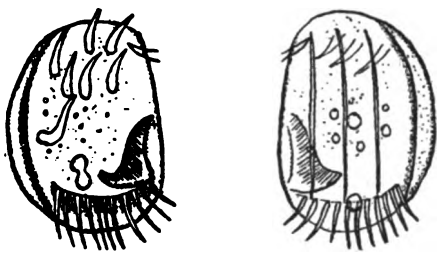
A. polystyla Stein.

A. polystyla Stein 1859 (subgenus Onychaspis).
Onychaspis Diesing 1865.
A. polystyla var. maxima Gourret et Roeser 1886.
A. plana Perejas. 1886.
A. polystyla Calkins 1902.

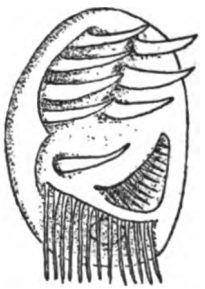
A. lynceus ventralside
Stein-Taf. III, Fig. 4

Body oval, the margin entire, the left border nearly straight; the dorsal surface slightly convex, traversed by three longitudinal furrows; ventral styles 7 to 8, forming two anterior, oblique, parallel rows of 3 or 4, and 3, one separate ventral style stationed by itself to the right and rear of the other six; anal styles variable 10-13. L—1/510". Sea water.

Distributions:—Warberg (Quenn), Bay of Concarneau (Fabre-Domergue) Harbor of Marseille and Bastia Corsica (Gour. et Roes.), Harbor of Triest (Stein), Gulf of Naples (Geta-Entz), Black Sea (Perejas.), Woods Hole, U. S. A. (Calkins).



A. polystyla—ventral and dorsal side.
Stein-Taf. III—Figs. 18 and 19.



A. polystyla—ventral side
Calkins—Fig. 57.

This species is evidently close to the preceding in all except the number and distribution of the cirri. In these it is very variable. Calkin's variety from Woods Hole differs from Stein's original form in the possession of 8 ventral and 13 anal styles—the former banked and very massive. A. plana as described by Perejaslawzewa is identical in all essentials with this species. A form described by Gourret et Roeser 1886 as A. polystyla var. maxima has 7 anal styles. The drawing (Pl. XXXIV, Fig. 1), which is evidently from the dorsal rather than the ventral side as stated, is so

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inaccurate that it is of little value. Except for the small number of anal cirri, the description is fairly close to that of Stein.

A. turrita (Ehr.)

Euplotes turritus Ehr. 1838.
Aspidisca turrita Clap. et Lach. 1857.

Body suborbicular, widest and somewhat truncate posteriorly, its marginal border even, the left side nearly straight, the opposite one rounded; a thorn-like recurved spine developed from the center of the dorsal surface; ventral and anal styles as in A. lynceus. L—1/450". Fresh and salt water.

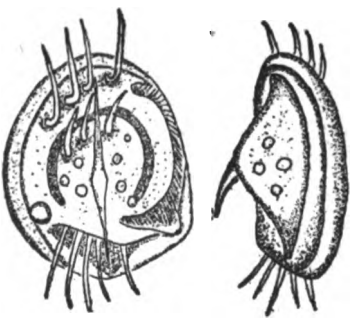
Distributions: Baltic (Ostsee) at Hapsal (Eichwald), Wismar (Ehr.) Gulf of Naples (Geta-Entz), Gulf of Mexico (Smith), common in pond water about New York City.

A. costata (Duj.)

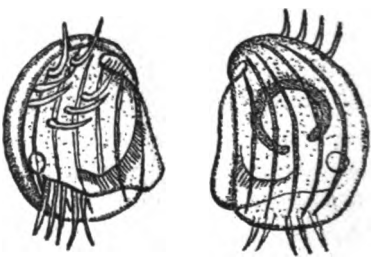
Trichoda cicada? Muller 1786, 1788.
Oxytricha cicada? Ehrenberg 1838.
Coccudina constata Duj. 1841.
Aspidisca cicada Clap. et Lach. 1857.
Aspidisca costata Stein 1859.

Body nearly oval, or left side nearly straight, right convex; the ventral portion developed outwards and backwards beneath the peristone in the form of a triangular plate toward the posterior extremity of the left side; dorsal surface grooved by six longitudinal furrows; ventral styles 7, in two oblique rows—anterior 4, posterior 3; anal styles 5. L—1/690". Brackish or pond water.

Distribution:—North Sea Water Aquarium (Fresenius), Finland Brackwasserbuchten at Ransösund (Levander), Gulf of Genoa (Gruber), Gulf of Mexico (Smith), Buenos Aires (de la Rue), common in pond water in vicinity of New York City.



A. turrita—ventral and side view. Stein-Taf. III—Figs. 11 and 14.



A. costata—ventral and dorsal view. Stein-Taf. III—Figs. 15 and 16.

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This is the only species recognizable from Dujardin's drawings. It is there drawn with seven anal cirri, and the ventral plate is not shown. In spite of these inaccuracies, however, we must agree with Stein that the form of the body and the grooved dorsal surface are unmistakeable.

B. Left border armed with a single backwardly directed spur in its posterior third.

A. hexeris Quenn.

A. hexeris Quennerstedt 1867 v. 4.
A. Andrewii Mereshkowsky 1878.
A. hexeris Calkins 1902.

Body elliptical, about one and one-half times as long as broad, equally rounded at each extremity; the left border armed close behind its center with a single backwardly directed spurlike projection; dorsal surface usually grooved by three faint longitudinal furrows; ventral styles short and thick, 7 to 8 in number, generally in two rows of 3 and 3, with one placed by itself posteriorly, 6 anal styles. L—1/500". Salt water.

Distribution: Wisby, Gotland (Quenn), White Sea (Meresch), Woods Hole, U. S. A. (Calkins).

A. hexeris as described by Calkins from Woods Hole agrees closely with Quennerstedt's original description except for the possession of 8 ventral styles. As already indicated this character is not believed to be of importance in differentiating species. A.

A. hexeris—dorsal side.
Quenn. II—Fig. 19.

A. hexeris—ventral side.
Calkins—Fig. 50.

Andrewii Meresch. is in all respects identical with this species. Hamburger und von Buddenbrock 1911 consider this species to be identical with A. leptaspis Fresenius. The resemblance between the two is merely superficial, however, for the anterior spur and

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the marked serration of the cuirass, both of which are extremely constant in A. leptaspis, are never found in A. hexeris either in the European or American varieties.

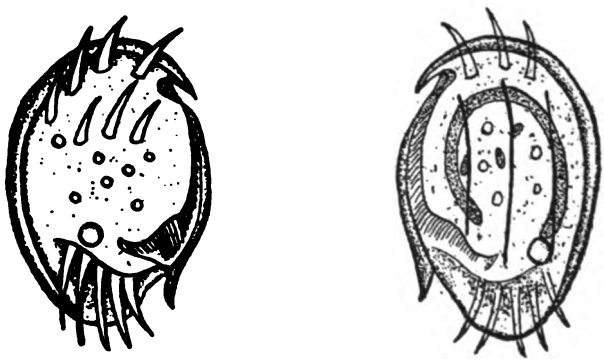
C. Left border armed with two spurs, one in the anterior and one in the posterior third.

A. lyncaster (Muller).

Kerona lyncaster? Muller 1786.
Trichoda lyncaster Muller 1788.
A. lyncaster Stein 1859.

Body ovate, rounded on the right side and at the two ends, the anterior border of the left side incised so as to produce a posteriorly directed spur-like projection, a similar aculeate spur, curved slightly outwards, projecting from the ventral surface of the carapace on the same side within a short distance of the posterior margin; ventral styles short and thick, 7 in number, placed in two oblique rows of 4 and 3; anal styles 5. L—1/450′—1/360″. Salt water.

Distribution: Finland See at Löfo (Levander), Baltic Sea at Travemünde and Stralsund (Stein), Kiel Harbor (Möbius), Gold of Genoa (Gruber), Gulf of Naples (Geta-Entz), Harbor of Bastia Corsica (Gour. et Roes.)



A. lyncaster—ventral and dorsal view.
Stein-Taf. III—Figs. 1 and 2.

A. leptaspis (Fresenius).

A. leptaspis Fresenius 1865.
A. crenata Fabre-Domergue 1885.

Body oval, blunt at either end, the left side somewhat less curved than the right; the right border smooth, the left incised in the anterior third so as to produce a feeble spurlike projection; in its posterior third a similar but more prominent and sickle shaped spur from both the upper and lower plates of the cuirass; outer posterior border of upper plate evenly notched or ser-

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rated; lower plate similarly serrate anteriorly and posteriorly; upper surface may show three feeble longitudinal striations; ventral styles 7—two rows of 3 each, with one placed posteriorly by itself; anal styles 5 to 7. L—.065—.07 mm. Salt water.

Distribution: North Sea Water Aquarium (Fresenius), Osterschelde, Belgian coast (Rees), Bay of Concarneau (Fabre-Domergue).

This peculiar form is of especial interest because of the observation of van Rees 1884 with regard to the fraying out of one of the anal cirri so as to form three, making a total of seven instead of five as the species was originally described by Fresenius. The seven styled form was described by Fabre-Domergue 1885 as a new species A. crenata. The fact that such a condition occurs proves that the actual number of cirri is of little value in recognizing species, and suggests that a similar condition may exist in other Hypotrichs which have not been observed for an extended period. The anterior tuft of cilia figured by van Rees is also of interest. If the observation is correct it appears that the adoral cilia may sometimes extend a considerable distance towards the anterior end. On the other hand it seems more likely that the tuft represents an additional ventral cirrus—a condition observed by Calkins in at least two other species of Aspidisca.

A. leptaspis—ventral view.
van Rees—Fig. 11.

Ventral view
Fabre-Domerque—Fig. VII.

D. Left border with three spurlike projections.

A. sedigita Quenn.

A. sedigita Quennerstedt 1868 v6.

Body broadly ovate, suborbicular, the right border smooth and rounded, the left one incised so as to produce an anterior, middle, and posterior spurlike projection—the middle spur extending from the ventral surface as does the posterior one in A. lyncaster; ventral styles 7, short and thick, centrally

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disposed; six stout, scarcely prominent anal styles. L—1/325". Salt water. Distribution: Wisby, Gotland (Quenn).

This species is made identical with A. lyncaster by Hamburger und von Buddenbrock 1911. According to Quennerstedt's original description, however, A. sedigita possesses a third spur at the posterior end of the body giving it a characteristic pointed appearance. This together with its smaller size makes it necessary that the species be retained.

A. sedigita—Dorsal side.
Quenn-Taf. II—Fig. 2.

Doubtful Species

A. denticulata Ehr. 1838 is insufficiently described and the figure is not recognizable as an Aspidisca. The name suggests that it may be identical with A. leptaspis Fres. but the description of the denticulations as on the left side precludes this.

A. radiata Fromentel and A. pulvinata Fromentel 1874 probably cannot be considered good species because of insufficient description. The former has ten ventral cirri and the adoral fringe of cilia at the anterior end, which probably put it outside the limits of the genus. The latter has no apparent differences from A. costata (Duj.).

A. bipartita Gourret et Roeser 1886 very clearly has none of the characters of the genus, such as a group of ventral styles and an adoral fringe of cilia on the left side of the ventral surface. The description and pictures look very much like a minute crustacean belonging to the group Cladocera.

Columbia University, New York City.
October 19th, 1916.

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NEMATODE TECHNIQUE*

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Difficulties in making satisfactory preparations for examination have prevented many from taking up problems with nematodes. The author, in his study on some of the parasitic species, undertook to solve a few of the general problems in the technique and also some of the more particular processes for demonstrating certain organs and systems. This work has by no means exhausted the problem, but perhaps the methods given here will serve as a guide for future work along this line. These methods, while applicable to free-living nematodes, have been worked out in particular for the parasitic forms.

Collecting parasitic nematodes differs little from collecting other parasitic worms. However, there are some things that have to be kept carefully in mind. In the first place many forms are encountered with such well developed mouth parts that it is difficult to free them from their attachments without injury. The best method is to take hold of the host tissue very close to the mouth of the parasite with a pair of fine-pointed forceps and with gentle pressure and slight traction, to pinch the animal off; in this procedure the forceps take up so little tissue that when the worm is freed they close with nothing between their points. When the worms are not so firmly attached, by gently stroking them with a fine camel's-hair brush in a direction away from the tissue, they will often come loose; such a brush will be found in general to be the most convenient instrument for handling parasitic nematodes.

In looking for alimentary parasites it is a safe plan to split the gut with a needle, for then the danger of cutting a worm is nil; however, in the examination of the larger animals this is not always practical and here one must very cautiously cut the wall with blunt scissors. In most cases gripping the opened gut

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tightly between the two prongs of the forceps and drawing it thru them will free, in good condition, all of the parasites that adhere. If an examination is to be made of the lungs, liver, kidneys or muscles of an animal it will be found of advantage to tease them out with needles. In case nematodes are found encysted, they should be carefully dissected out, before preservation, under a good lens or binocular.

Most nematodes are rather sensative to changes in the osmotic condition of the medium in which they are placed. Tap water has been found to be far better for temporary keeping than physiological salt solution, and while some of them live for a while in distilled water, this medium should be generally avoided. For most of the nematodes found in fresh-water fishes a 0.3% salt solution will be found to be about right. As a matter of fact the best medium to keep them in while making the necessary observations on the living material is that which collects in the dish in which the examination is being made, and into which the isolated organ has been placed for examination. In spite of the fact that Looss found the Sclerostomidae of horses and donkeys remain in good condition after being kept for hours in physiological salt solution, it will be found that there is an advantage in killing and fixing the worms as soon as possible after they have been removed from the host. Under no condition should they be allowed to dry at any stage in the process.

Owing to the nature of the cuticula nematodes are very hard to preserve. Most cold killing fluids penetrate so slowly that nematodes live for hours in fluids which will kill other parasitic worms in a few seconds; the hot fluids coagulate the proteins of their bodies before they get in, thus making penetration harder, but they have the advantage over the cold reagents in that the specimens are killed in a straight position. While this last statement is in general true, it does not always hold, for with most of the Trichinellidæ the anterior region of the body will coil up like a spring even tho the fluid be fairly hot. This may be prevented in a great measure, if not entirely, by placing the anterior end of the worm in the angle formed by pressing the points of a pair of forceps together and working the specimens to and fro in the hot fluid as

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soon as placed into it. In applying this method the fluid should be first heated and then the worms transferred into it. In most species the posterior region of the males curl up, and no method has yet been devised to prevent this. No successful method of anesthetizing the worms before fixation has been found by the author, and this procedure should be avoided. If worms are cut into pieces before fixation the organs crowd out at the cut end and the whole animal presents a very abnormal picture when sectioned, due to the pulling and stretching of the tissues. However, for certain purposes this is not an objection.

Without doubt the most successful killing fluid ever recommended for general work in this group is the one devised and used by Looss. Because of the simplicity of its use and general good results, it has been followed by nearly all workers since he (1901) published the method. The procedure is this: worms, carefully freed from debris, are placed in a mixture of 20% glycerol in 70% alcohol, if the worms are small, and in 10% glycerol and 70% alcohol, if they are large, the mixture previously being heated to 80° C. They may be preserved in this mixture for future use or put into an incubator or on a paraffin bath with the cover off of the dish in which they are contained. The alcohol and water are allowed to evaporate slowly from the glycerol, care being used to keep the worms from coming into pure glycerol too quickly, else they will collapse.

Twenty-four hours is slow enough for some small worms, but twenty-four days is too fast for some of the large ascarids. The time may be regulated by the temperature or by covering the dish partially. In glycerol they are very transparent and may be studied or kept in this fluid or transferred directly into glycerine-jelly and mounted on a slide. Of course they are not stained by this process, but many characters come out beautifully in material prepared in this way. Looss transfers material directly into 96% alcohol for sectioning, after making a few incisions in the cuticula with a very fine sharp knife. They are then brought into absolute alcohol, oil of cedar and finally paraffin. He found that oil of cedar was the best medium for this purpose; on the other hand the author has had no success with it, whatsoever, but since the product as

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sold commercially varies so greatly, no importance can be attached to his negative results. In connection with the very excellent work of Looss it is interesting, from the standpoint of what is to follow, to quote one of his statements: "Canada-balsam was almost entirely excluded because it made unstained objects too transparent, and stained nematodes were less favorable for mounting than unstained ones". On the same subject Braun and Lühe express themselves in no uncertain terms: "Man wirt von vornherein auf das Färben ganzer Tiere verzichten müssen und kann auch wegen der kaum zu vermeidenden Schrumpfung der Cuticula beim Aufhellen mit Kreosot, Terpentin, etc. den Einschluss in Canada-balsam oder anderen Harzen nicht anwenden".

As for general nematode technique, there seems to be in literature few references or results published other than this work of Looss, altho there are indications that other killing fluids were used to some extent by the authors prior to 1901. Since this time a method for handling nematodes was proposed by Langeron which can be used to advantage, since it is very rapid and reliable. Nematodes are killed in diluted formol (5:100) and then transferred, after several hours, into a lacto-phenol mixture, made as follows and used first in half strength:

Glycerol	2 parts
Phenol	1 part
Lactic acid	1 part
Distilled water	1 part

After a few hours the nematodes are placed in this fluid of full strength and either preserved in it, or mounted on a slide in a drop of the mixture, enclosed in a gold-size ring, covered with a cover glass, and sealed.

Looss and Braun and Lühe claim that it is not practical to mount nematodes in balsam after staining, yet when material is handled in the correct manner, it becomes the very best medium and further allows of staining to suit the needs of the student. It is obvious that animals so sensative to changes in osmotic pressure will require very cautious treatment while bringing them into paraffin or balsam, and it was Cobb who first proposed a plan by which they could be brought into these reagents, and for this purpose he

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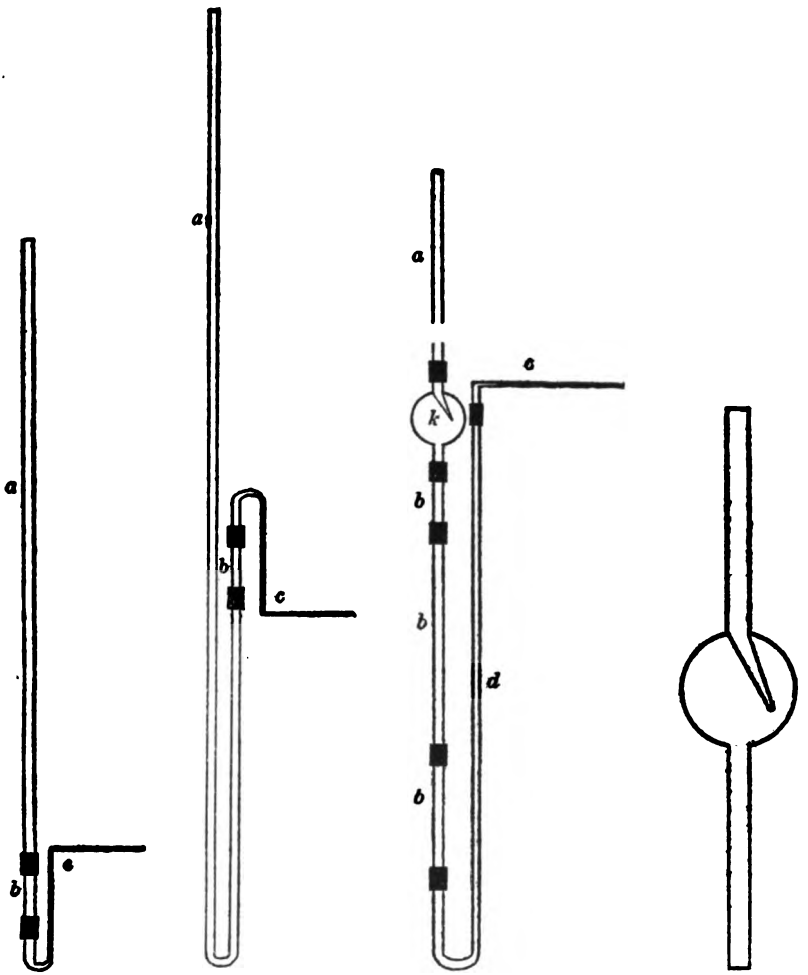

FIG. 1. FIG. 2. FIG. 3. FIG. 4.

FIG. 1. Differentiator used for dehydration. *a*, reservoir; *b*, object box, plugged at each end with cotton; *c*, filter, also with cotton plug. (After Cobb.)

FIG. 2. Differentiator used for transferring objects from absolute alcohol into a clearing fluid. Letters as in Fig. 1. (After Cobb.)

FIG. 3. New type of differentiator for dehydrating. *a*, reservoir; *b*, object holders; *c*, filter and regulation devise; *d*, safety tube; *k*, mixing chamber. The reservoir should be two metres long, and is shown sectioned in the figure. In filling, avoid bubbles.

FIG. 4. The mixing chamber of the new differentiator. Made of glass tubing, in which a bulb is blown and a pointed piece of tubing is fused into one end.

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devised the differentiator. This ingenious devise (Fig. 1) consists of a glass tube 5 mm. or more in diameter, which is used for the reservoir. To this is attached, by rubber tubing, the object box, a short piece of tubing, plugged at each end with a cotton plug and holding the specimens. The object box is also attached to the filter, a third piece of glass tubing, which is drawn out into a fine capillary tip and bent as shown in the figure. The filter is filled with whatever fluid the worms are in, for instance corrosive sublimate solution, and is attached to the object box, after inserting a cotton plug in the proximal end. The box is filled and the object placed into it, the distal plug inserted and the whole connected to the reservoir, which is filled in the following manner: mix equal parts of sublimate solution and 35% alcohol (solution 2), equal parts of solution 2 and sublimate solution (solution 1), equal parts of solution 2 and 33% alcohol (solution 3). The reservoir if filled one-fourth full of each solution in order, 1, 2, 3, and then on the top 33% alcohol is added to fill the reservoir. A wire is passed down the reservoir to cause a little mixture, and withdrawn. Minute drops flow from the filter and the rate of flow can be regulated by tilting the differentiator, so that the worms are brought into 33% alcohol in from two to five hours. As the reservoir is emptied it is filled with solutions of the next higher grade of alcohol made up in the manner described. Specimens may be stained here, destained, and finally brought into absolute alcohol. Because the clearing fluids are heavier than alcohol it is necessary to use a reverse form at the tip of the differentiator. (Fig. 2). Here the pressure is equalized by bending the reservoir as figured and by the same general plan the worms are brought into the clearing fluid (Cobb used oil of cloves if the worms were uncut and chloroform if they were cut) and finally into balsam.

For some time this method was used with a fair amount of success but certain disadvantages attended it. The most obvious one is that in using the instrument so much time is required in the making up of the many solutions and the constant refilling of the reservoir. Then, too, the change is not gradual enough for the best work, especially with some very delicate worms. Further, clearing fluids attack the rubber connections. After many

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trials the following method and instrument for handling the worms were devised: worms are killed in 50% alcohol heated to 60-75° C. and transferred at once to any one of the following mixtures: (A) Carnoy's (33 parts chloroform, 33 parts glacial acetic acid, 33 parts alcohol, the mixture is then saturated with corrosive sublimate), (B) a mixture made of equal parts of alcohol, water and acetic acid, saturated with corrosive sublimate, or best of all (C) using the latter mixture with the addition of enough osmic acid to make the solution contain from 0.05% to 0.1% osmic acid. These killing fluids give very good results, but for the very fine preservation of histological detail, the last one given is by far the best. In these fluids the material is left for from one to ten hours, depending upon the size of the worms. It is possible to get good results by killing in hot water and then transferring into a saturated aquous solution of corrosive sublimate, with or without acetic acid, or in combination with osmic acid. If the nematodes are killed in osmic mixtures they should be bleached by adding a little hydrogen peroxide to the water into which they are brought after fixation in water solutions, or to 50% alcohol if they are killed in alcoholic solutions. After this treatment they are brought very gradually into 70% alcohol and the corrosive sublimate removed with iodine solution. One can usually succeed in doing this within a period of ten or twelve hours and six or eight changes, so that the nematodes are perfectly round and not distorted, but to be safe, the new differentiator (Fig. 3) to be described later should be used. After the iodine is removed they are graduated into 80% alcohol and preserved in this strength, being careful not to let it become weaker. If they are to be used for totos at once they should be stained in Ehrlich's acid hematoxylin (diluted 1:25-50) or in Delafield's hematoxylin of the same dilution, in which event it will be a saving of time to remove the corrosive sublimate when they are in water or 35% alcohol with iodine dissolved in water to which a little potassium iodide is added. For sectioning it is useless to try to stain in toto, yet material may well be stained in one of the hematoxylins in order to make them easy to see and handle, or Mayer's paracarmin may be used for this purpose when they are in 70% alcohol. In either case they are stained for twenty-four hours

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and then destained to the proper intensity in 5% hydrochloric acid in water or 35% alcohol and the hematoxylin material "blued" by transferring into a 5% solution of ammonia water, in either water or 35% alcohol. They are then ready for the differentiator. The new form of this instrument (Fig. 3) consists of the following parts: the reservoir, the mixing chamber, the object holders, the safety tube, and the filter and flow regulation device. The reservoir is a shell tube with an inside diameter of 5 mm. and two meters long, so that it will hold about 45 cc. of alcohol. The mixing chamber (Fig. 4) is made in the following manner: A piece of good thick-walled glass tubing, with an inside diameter of 4 mm. is pulled out at one end and sealed. Ten centimeters from the sealed end the tube is heated in a good flame and a bulb blown that will hold between six and eight cubic centimeters, then the sealed end is cut off as near the bulb as possible. The end that has been drawn out is bent in the flame near the tip (which has been broken off) at a slight angle and inserted into the bulb and the two held so that the tip points to one side and a little below the middle of the bulb; in this position they are fused together, and both ends of the tube are cut and rounded off about 5 cm. from either end of the bulb. If for any reason this type of mixing chamber cannot be made, the substitute shown in Figure 5 can be used. It is made with a piece of large glass tubing and rubber stoppers. The object holders are pieces of shell tubing with an inside diameter of from 5 to 7.5 mm. and varying in length from 5 cm. to that needed to hold the worms without bending. These are plugged with cotton at either end to keep the worms in place. The safety tube is 2 or 3 mm. inside diameter and bent in the shape of a U with one arm 5 mm. long and the other long enough to reach to the middle of the mixing chamber when in use. The filter is a tube 3 mm. in diameter, bent in the form of a right angle and pulled out into a fine capillary at one end; a cotton plug in it acts as a filter to prevent stoppage by particles in the alcohols. Rubber tubing is used for connections. To manipulate the instrument requires some practice and the order is essential. The filter is filled and connected to the safety tube as shown in Figure 3. A pinch-cock on a piece of rubber tubing at the end of the safety tube will keep it full while the rest of the

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apparatus is being filled with the grade of alcohol or water in which the specimens are. The object holders are next filled and the specimens put into them and plugged with cotton. As many holders can be filled as necessary and connected together in a long line, each being properly labeled. These are then connected to the safety tube and a pinch-cock applied to the rubber-tube-capped free end. The mixing chamber is capped with a short rubber tube, then filled by drawing in the liquid of the same strength in which the specimens are, whereupon it is closed with a pinch-cock. This is then connected to the terminal object holder, the other end of the mixing chamber being connected to the reservoir. The reservoir is then filled by putting in four grades, one on top of the other, in order,

FIG. 5.

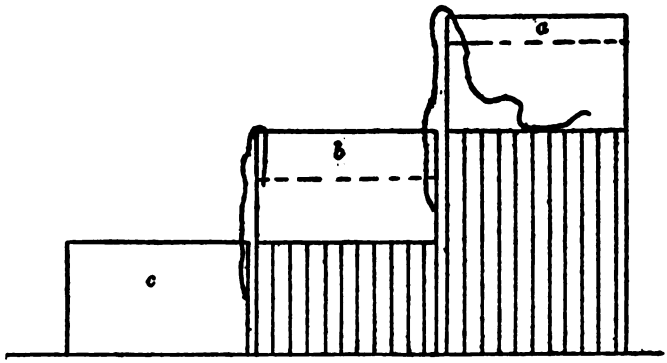

FIG. 6.

FIG. 5. Mixing chamber made of a piece of glass tubing with rubber stoppers and inlet and outlet tubes.

FIG. 6. String siphon system for handling small embryos and eggs and transferring nematodes from absolute alcohol into a clearing fluid. *a*, for the clearing fluid; *b*, for the objects; *c*, waste. *a* and *b* are supported on wooden blocks. The whole is placed under a bell-jar with sulphuric acid in a beaker to absorb the moisture.

such as 35%, 50%, 70%, 85% alcohol, the lowest being always the next step above the fluid in which the worms are contained. The capillary tip is regulated to yield 5 to 10 small drops per minute, and as the reservoir is emptied, it is filled with 95% alcohol until 20 cc. have been added. It is then filled with absolute alcohol until 50 cc. have been added, whereupon the apparatus is allowed to empty itself

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as far as the safety tube will permit, as this will never allow it to run dry. The mixing chamber will attend to the mixing of the alcohols as the lighter one is forced under the heavier one and mixes very efficiently. After the absolute alcohol is all in the reservoir a calcium chloride tube is attached to the top of the reservoir, and the specimens allowed to remain in the alcohol for several hours. The object holders are now taken out ready for the clearing fluid.

The best method for getting the material into the clearing fluid is by means of a string siphon. Three Stendor dishes are arranged in stair-step fashion (Fig. 6). The objects are placed in the middle dish, either in the tubes or free, and this dish is connected with the receiving end of a siphon, made by using any suitable piece of string, regulating the flow by the size of the string. From this dish another string is led to the waste dish below. If the nematodes are to be used for sectioning one puts in the upper dish a mixture of absolute alcohol and xylol, half and half, and after this has run out xylol full strength, regulating the flow so that the specimens come into pure xylol in about 36 hours. If the specimens are intended for toto mounts synthetic oil of wintergreen (methyl salicylate) is used in the same way as xylol. The xylol-cleared worms are gradually brought into paraffin either after being cut into pieces or still whole, by allowing small pieces to dissolve in the xylol; they are finally saturated in xylol-paraffin at 35° C., and embedded after infiltration for one-half to one and a half hours in paraffin melting at 56° to 58° C. By coating, with soft paraffin, the block of hard paraffin in which the nematodes are embedded, it will be found that good sections in series can be made, provided the knife is kept sharp. For this convenient method, the author is indebted to Mr. H. G. May. Some little success has been met with infiltration in vacuo and this method, if adapted somewhat, will undoubtedly yield results worth while. The worms cleared in oil of wintergreen should be put in parchment boxes or boxes made of heavy linen bond paper and placed in Stendor dishes with damar dissolved in oil of wintergreen, and the mounting medium allowed to slowly dialyse and penetrate. It is best to pierce the cuticula in one or two places before the worms are placed into the cups, and this can be done with a sharp needle so that none of the internal organs are injured.

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After twenty-four hours treatment in this manner they may be mounted under a cover glass on a slide in damar in oil of wintergreen. For staining sections good results are obtained with Delafield's hematoxylin, Ehrlich's acid hematoxylin, Unna's orcein method, Mallory's connective tissue stain and thionin in saturated solution in 1% phenol. Borax carmine is of no value and strange as it may seem, iron hematoxylin yields also poor results.

It will often be found of advantage to dissect out parts of nematodes and for this purpose glass rods drawn out into fine points are useful. For dissecting out the mouth parts, maceration in concentrated potassium hydroxide, and mounting in glycerine-jelly will give good results.

Nematode embryos and eggs are killed in strong Flemming's or the fluid of Ripart and Petit (camphor water, not saturated, 75 gms., distilled water, 75 gms., glacial acetic acid, 1 gm., copper acetate, 0.3 gm., copper chloride, 0.3 gm.) to which a few drops of osmic acid have been added, and are either carried thru the staining and dehydrating process in a differentiator or in a string siphon system. In using the latter it is best to cover it with a bell-jar and enclose also a beaker of sulphuric acid to absorbe the moisture.

Goldschmidt found that the nerve technique of Bethe, Apáthy, Wolff-Bielschowsky and Cajal could be applied to nematodes as well as to other forms. He offered a modification of the Cajal method that gave good results. Material is fixed in ammonical alcohol for 24 hours, 6 days in 10% silver nitrate in an incubator, 24 hours in hydrochinon or pyrogallol and formol; it is then embedded and sectioned. The sections are treated for twenty minutes in 0.1% goldchloride and reduced in a sodium fixing bath for half an hour. His method of preparing toto mounts of the large forms to show the nervous system was to split the cuticula and remove the esophagus. The stretched out "shell" was then stained for 6 to 8 hours in Nissl's alkaline methylene blue at 60° C., dehydrated and cleared in oil of cloves, in which they were kept, the excess stain being taken out by the action of the oil of cloves in two or three days.

The methods herein described have given good results for the author and it is hoped that others will be able to reproduce and

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improve upon them. Several failures should not discourage any one, for at best time is required to obtain really good results in this very difficult field.

The author wishes to express his sincere thanks to Professor Henry B. Ward for his careful suggestions and criticism of the manuscript of this article. Valuable assistance in many ways has been received thru the close personal association with my friend Mr. Henry G. May.

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DEPARTMENT OF NOTES. REVIEWS. ETC.

It is the purpose, in this department, to present from time to time brief original notes, both of methods of work and of results, by members of the Society. All members are invited to submit such items. In the absence of these there will be given a few brief abstracts of recent work of more general interest to students and teachers. There will be no attempt to make these abstracts exhaustive. They will illustrate progress, without attempting to define it, and will thus give to the teacher current illustrations, and to the isolated suggestions of suitable fields of investigation.—[Editor.]

ENTOMOLOGICAL NOTES

Collembola.—Folsom ('16, Proc. U. S. Nat. Mus., 50:477-525) presents a paper treating of all of the known species of North American *Poduridæ (Collembola),* with the exception of the subfamily *Onychiurinæ*. Keys to all of the taxonomic groups and full descriptions of the species are features of the paper. A bibliography of one hundred seventeen titles, and eighteen plates containing two hundred fifty figures, add to the value of the work. It is a paper indispensable to persons interested in the *Collembola.*

Pure Lines in Aphids.—Ewing ('16, Biol. Bull., 31:53-112), in a paper entitled "Eighty-seven Generations in a Parthenogenetic Pure Line of Aphis avenæ Fab.", finds no summation effect by selection, using six different fluctuating variations, and is of the opinion that summation through continued selection is not to be expected since fluctuations in such a pure line "are in general not dependent upon germinal variations." Selection from extreme variants was without effect on somatic characters of succeeding generations. Long continued selection from the extreme variant in each succeeding generation produced no more change in the mode of the variable than did the selection from individuals differing but slightly from the mean of the line and for only a few generations. Nor was the mean of the line shifted when selections from extreme variants for forty-four successive generations were made, using a character (body-length) "which is known to be inherited in the higher animals that reproduce sexually." Variations in body-length were found to be largely due to variations in temperature and food. The rarely appearing discontinuous variations were apparently not inherited. Size, fecundity, and color were not affected by long continued parthenogenetic reproduction. Pædogenesis occurred occasionally

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among the nymphs of both winged and wingless forms and the offspring which reached maturity were normal. It is thought that pædogenesis in this aphid is due to "the arrested development of the body in general, while the reproductive organs become completely functional."

Insect Flagellates and Disease.—Fantham and Porter ('16, Journ. Parasitology, 2:149-166) have studied "the significance of certain natural flagellates of insects in the evolution of disease in vertebrates." Flagellates, including members of the genera *Herpetomonas* and *Crithidia*, secured from the alimentary tracts of several species of insects, were introduced into representatives of the *Pisces, Amphibia, Reptilia, Aves,* and *Mammalia,* by inoculation or by feeding. The latter was accomplished by feeding the host with the infected insects, or with the intestines of the insects, or with food contaminated with the feces of insects containing the resistant stages of the *Flagellata* concerned. It was found by these methods that *herpetomoniasis* can be induced in various warm and cold-blooded vertebrates, the nature of the infection and the protozoan parasites found in the vertebrate hosts resembling those of human and canine *leishmaniases. Herpetomonas jaculum, H. stratiomyiæ, H. pediculi, H. ctenocephali, H. culicis,* and *Crithidia gerridis* were found pathogenic to warm-blooded vertebrates, the first and last mentioned having been also successfully introduced into cold-blooded hosts, such as fishes, frogs, toads, lizards, and grass-snakes. In acute cases of the induced disease, the flagellate form of the parasite was most numerous at the death of the host, while in chronic cases the non-flagellate forms predominated. These writers hold that a *Leishmania* is morphologically a *Herpetomonas,* that *leishmaniases* are invertebrate-borne *herpetomoniases,* "and that these maladies have been evolved from flagellates of invertebrates (especially insects), which have been able to adapt themselves to life in vertebrates."

Phylloxera Galls.—Rosen ('16, Am. Journ. Botany, 3:337-360) finds that the leaf-gall produced by *Phylloxera vastatrix,* which occurs on *Vitis vulpina,* starts to develop on the embryonic bud leaves and soon produces a depression due to the upward growth of tissue at the sides. That portion of the leaf surrounding the proboscis and beneath the insect shows no proliferation.

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Gall development depends upon leaf development, the gall becoming mature when the leaf reaches maximum size. No support for the theory that the insect causes gall formation by the injection of some chemical appears in this study. The initial stimulus for gall development is believed to be the continuous sucking action of the insect at a single fixed point.

Color Inheritance in Phasmidæ.—MacBride and Jackson ('15, Proc. Royal Soc. London, (B) 89:109-118) have studied the inheritance of color in a phasmid, *Carausius morosus,* from southern India, which exhibits marked color varieties. Of the several thousand insects reared, only six males and one gynandromorph appeared, the results therefore being concerned with parthenogenetic inheritance. All the insects are alike at hatching, having a definite color pattern of green and brown pigments. In subsequent growth, the green pigment overpowers the brown so that the insect appears pure green in the majority of cases, although, in about three per cent., the reverse occurred. The color of the mother does not influence the proportion of predominantly green or predominantly brown offspring. Pure green forms were secured from larvæ reared in complete darkness. Conditions of reduced light did not increase the proportion of brown forms. Males are very rarely produced from the eggs of unfertilized females.

Cuticula vs. Parasites.—Thompson ('15, Proc. Cambridge Philosoph. Soc., 18:51-55) presents argument and data in support of "the cuticula of insects as a means of defence against parasites." Some of the positive points stressed are: (1) Thickness and resistance of the cuticula sometimes effectively prevent the entrance of dipterous parasites. (2) In the process of molting, the larvæ of insects often succeed in freeing themselves from the unhatched eggs of dipterous insects. (3) Certain parasites, which have made entrance into the body of the host but have not withdrawn completely into the body-cavity, are thrown off in molting. (4) The probability that the cuticula is some protection even against the well-developed ovipositor of hymenopterous parasites. It is contended that the marked parasitism to which insects are subject is not necessarily a proof of the inefficiency of the cuticula and a possible explanation can be found in the fact that a great many of the parasites

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are themselves arthropods and that the arthropod structure and life history render the members of the group especially able to support parasitic invasions. "It seems highly probable that the cuticular armour, and the function of ecdysis correlated with it, in reality arrests a very considerable part of the violent attack which many members of the Arthropoda are obliged to sustain."

Polyhedral Bodies.—Glaser and Chapman ('16, Biol. Bull., 30:367-390) have studied the nature of the curious crystal-like structures called *polyhedral bodies* or *polyhedra* which are constantly associated with certain diseases of insects, known in America by the vernacular name *wilt*. The larval stages of thirteen species of *Lepidoptera*, belonging to eight different families, have been found to be susceptible to the polyhedral diseases, and at certain times these diseases kill off from 30 to 70 per cent. of some of the most noxious pests (gipsy-moth, tent-caterpillars, and army-worms). These structurally complicated polyhedra, which arise in the nuclei of certain tissue cells, are specific for a certain type of disease. They are "nucleoprotein crystal-like degeneration-products and not organisms."

Photosensitivity of Blowfly Larvæ.—Patten ('16, Journ. Exp. Zool., 20:585-598), in studying the changes of photosensitivity with age in the larvæ of *Calliphora erythrocephala*, tested the specimens daily, from hatching to pupation, by subjecting a larva, crawling under the influence of a horizontal beam of light, to an instantaneous change of 90° in the direction of the beam and measured the resulting change in the direction of locomotion. The curve of photosensitivity, constructed on individual averages, showed constant negative reaction and that increased amplitude occurred during the first days of larval life, the maximum of 81° being attained on the fourth day. Steady decrease followed until the seventh day and thence to pupation the amplitude remained almost constant. Decrease in sensitivity was coincident with the initiation of the migration period.

Chemotropic Response of House-fly.—Richardson ('16, Science, 43:613-616) experimented with a number of organic and inorganic compounds which occur as products of fermentation in barnyard manures in an effort to discover whether the distinct oviposition

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preference of the house-fly for horse
some volatile chemical substance whi
during the early stages of decomposi
be a strong alluring agent and was
females. In experiments with acid
sponse was approximately in inverse
source of the ammonia. Butyric ac
ianic acid, augmented the oviposition
ammoniated cotton. Ammonium ca
ing these acids produced no response
in barnyard manure, the evidence p
agents for the fly.

Aquatic Lepidoptera.—Welch
9:159-190) reports on the biology
(Nymphula maculalis and *N. icciusal*
invariably deposited about the egg
(Donacia) in the floating leaves of t
tory experiments showed that in the
egg masses of *N. maculalis* may b
about the leaf margin or artificial
orientation of the eggs in a mass is d
gills are absent in the first instar but a
number of gill filaments per larva inc
instar to over four hundred in the f
of cases from excised pieces of food
activity, these cases functioning as
water. Larval dissemination is accor
tary propulsion in detached cases, b
currents on detached cases, and ind
other aquatic insects which separate
from the petiole. The larvæ and pup
ence under water. The adult is a
N. icciusalis are laid on the margi
natans and independently of the acti
Tracheal gills are absent in all inst
that of *N. maculalis,* is a normal act

Syrphidæ.—Metcalf ('16, Main

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in a study of the *Syrphidæ* of Maine, gives particular attention to the larvæ of these flies, treating of the structure and habits in considerable detail. Five different structural types of larvæ occur, the species of each having approximately the same habits: (1) the aphidophagous type, which is composed mainly of predaceous species; (2) the boring type, which includes the species that feed in the bulbs of living plants; (3) the short-tailed, filth-inhabiting type, which includes a number of species that feed on exposed decaying animal and vegetable matter; (4) the long-tailed, filth-inhabiting type, which is characterized by an elongate, posterior, flexible, telescoping, respiratory process at least half as long as the body; includes a number of forms which are scavengers in habit; and (5) the microdon type, includes those anomalous forms, sometimes mistaken for *Mollusca* and *Coccidæ*, which live in the nests of ants. Descriptions of the life stages and the life histories of the species of Maine are given and the beneficial and injurious habits of the larvæ discussed. Keys to the known larvæ and pupæ of *Syrphidæ* are included. Nine plates include a large number of figures of structural detail and life history stages.

Nematode Parasites.—Merrill and Ford ('16, Journ. Agr. Research, 6:115-127) found two new species of *Nematoda* parasitic in insects. *Diplogaster labiata* parasitizes the adults of *Saperda tridentata (Coleoptera)*, infesting the digestive tract in such large numbers that they rupture the wall, escape into the body-cavity of the host, and cause its death. These nematodes were reared in water cultures to which macerated beetles were added as food, thus affording opportunity to work out the life history. Another nematode, *Diplogaster ærivora*, was found infesting the heads of termites *(Leucotermes lucifugus)* in numbers as high as 75 per host. They were found in the soil about infested termite colonies; also in the dead bodies of termites and other decaying matter. This parasite was successfully introduced into the termites. In cases of heavy infestation, the mortality of the host was high. *D. ærivora* was also reared in water cultures and the life history determined.

Cestodes in Musca domestica.—Gutberlet ('16, Journ. Am. Vet. Med. Assn., pp. 218-237) has carried on experiments which show that the cysticercoid stage of *Choanotænia infundibuliformis*,

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a cestode which infests chickens, occurs in the common house-fly *(Musca domestica)*. Flies which fed on the eggs of this tapeworm developed the cysticercoid stage and chickens fed on flies developed the adult worm, the identity of the two stages being determined by morphological comparison. Circumstantial evidence points to the probability that certain other insects which commonly occur about poultry yards and which are readily eaten by fowls are the intermediate hosts of other species of cestodes.

Spermatogenesis in Dragon-flies.—Smith ('16, Biol. Bull., 31:269-303) describes spermatogenesis in the dragon-fly, *Sympetrum semicinctum*, and for comparison has examined another dragon-fly, *Libellula basalis*. The maturing sex cells in the testes of the nymphs occur in globular cysts arranged, one or two layers deep, around a central duct which extends zig-zag through each organ. The cysts seem to have no definite arrangement in the tubule according to age but all of the developing stages of the spermatozoa may be found in a single transverse section. In both species, there are 25 spermatogonial chromosomes which are so closely crowded together that they are difficult to study. Apparently, the leptotene threads unite side by side to form a spireme which breaks up into segments that seem to open out along the original axis of synapsis to form rings. These rings condense into crosses and then into quadripartite bodies or prophase chromosomes. Twelve bivalent autosomes and one sex-chromosome occur in the primary spermatocyte. In the second spermatocyte division, the sex-chromosome passes to one pole undivided, thus giving rise to two kinds of spermatids and subsequently to two kinds of spermatozoa. In *Libellula basalis*, the sex-chromosome passes undivided to one pole in the primary spermatocyte division, thus forming two kinds of secondary spermatocytes, while in the secondary division, it divides equally. Two kinds of spermatozoa are also produced but by a slightly different process.

Insect and Mite Galls.—Wells ('16, Ohio Journ. Sci., 16:249-290) has made a survey of insect and mite galls on the hackberry *(Celtis occidentalis)*, giving particular attention to the histology of the galls and the gall bearing parts. The seventeen species of zoocecidia found were distributed as follows: *Acarinæ* 1, *Lepidop-*

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tera 1, *Hemiptera* 5, *Diptera* 10. All produce abnormal cell and tissue formations. The acarinous and lepidopterous galls are *kataplasmas* (cells and tissues differing but slightly from the normal), while the hemipterous and dipterous galls are *prosoplasmas* (cells and tissues differing fundamentally from the normal ones). The latter show definite specificity. Eight plates containing many figures of these galls and their morphological characters accompany the paper.

Viability of Mosquitoes.—Chidester and Patterson ('16, Ent. News, 37:272-274), in an experimental study of the influence of various concentrations of sea water on the viability of the salt marsh mosquitoes, *Aëdes sollicitans* and *Aëdes cantator,* find that under laboratory conditions the viability of the larvæ in salt water depends upon the salinity of the water from which they are taken and varies with the species. Larvæ of *A. cantator* died quickly in distilled water and in the higher percentages of salinity. All larvæ in water of 22 per cent. or above died within two days. Field records indicate that *A. sollicitans* lives and thrives in marsh water of a higher salinity than that which appears to be suitable for *A. cantator.* Evidence seems to indicate that the distribution and date of appearance of the two dominant species are, in part, dependent upon the salinity of the marsh water at various distances from the sea. It is suspected that investigation will show a certain amount of dissolved salt more favorable for the development of the eggs of one species than another.

Gregarines of Insects and Myriapods.—Watson ('16, Illinois Biol. Monographs, 2:1-258) reports the results of a study of the gregarines found as parasites in various *Orthoptera, Coleoptera,* and *Myriapoda.* Although primarily a work on gregarines, it contains much of interest to entomologists, since considerable attention was given to the relations of these parasites to their arthropod hosts. Twenty-two new species are described and additional data are given for many others. The paper includes a synopsis of the eugregarine records of the *Myriapoda, Coleoptera,* and *Orthoptera* of the world; also a list of the cephaline gregarines of the world and their hosts, followed by a second list arranged according to the hosts. Records of two hundred forty-three gregarines distributed among

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two hundred seventy-six hosts are given, these numbers including a few incomplete identifications. A valuable bibliography and fifteen plates containing three hundred thirty-eight figures are included in the paper.

Pupæ of Lepidoptera.—Mosher ('16, Bull. Ill. State Lab. Nat. Hist., 12:17-159) has presented an extensive paper on the "Classification of the Lepidoptera based on Characters of the Pupa." The external morphology of lepidopterous pupæ is worked out in detail. The paper is rich in analytical tables to the superfamilies, families, subfamilies, and genera. Full descriptions and discussions of the various groups are given. Attention was given to the phylogeny of the order, using the following characters as the basis: the number of movable segments; the freedom of the appendages; the number of sutures in the head; the relative length of the body segments; the presence or absence of visible labial and maxillary palpi; the presence of exposed portions of the prothoracic femora in specialized pupæ; and the method of dehiscence. The nature of the paper makes impossible a summary here, but it is the most comprehensive and connected study of lepidopterous pupæ which has appeared and forms an important basis for work on these quiescent stages.

Classification of Pupæ.—Mosher ('16, Annals Ent. Soc. Am., 9:136-158) reports on the classification of the pupæ of the saturniid moths *(Saturniidæ)*. The general characters of the pupæ of this family are described and a key to nine genera is given. Keys to certain species are also included. Detailed generic and specific descriptions are given for the pupæ of the following: *Copaxa lavendera, Telea polyphemus, Trophæa luna, Agapema galbina, Callosamia promethia, Callosamia angulifera, Eupackardia calleta, Rothschildia orizaba, Rothschildia jorulla, Samia californica, Samia cecropia, Samia columbia, Samia gloveri,* and *Philosamia walkeri.*

Breeding Habits of Orthoptera.—Turner ('16, Annals Ent. Soc. Am., 9:117-135) has made a survey of the breeding habits in the *Orthoptera* and finds that preliminary copulatory movements are, within narrow limits, constant for each group of this order but vary from very simple ones in *Mantidæ, Phasmidæ,* and *Acrididæ* to complex ones in *Blattidæ, Gryllidæ,* and *Locustidæ.* All males show sex discrimination but the females are aggressive and show sex

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discrimination only in some groups while in others they are entirely passive. Habits of copulation are typical for each family, e. g., in *Mantidæ, Phasmidæ,* and *Acrididæ* there is superposition of the body of the male; in *Blattidæ* and *Gryllidæ* superposition of the female occurs; and in *Locustidæ* end to end copulation is characteristic. Generalized reproductive behavior occurs in those families having the largest number of subfamilies. A significant parallelism between a classification based on reproductive behavior and one based upon palæontological evidence occurs, suggesting "that the different types of reproductive behavior have been fairly constant since their origin."

Brain of Termites.—Thompson ('16, Journ. Comp. Neurology, 26:553-603) has made a study of the brain of *Leucotermes flavipes* (termite) in the different castes, with reference to its finer structure, making, in addition, a comparison with corresponding organs in the castes of true ants. This comparison is interesting since both termites and ants have a complex social organization but differ in degree of specialization and intelligence. The study included the nymphs of the first and second form, the soldier, the worker, and the true adult. No sex differentiation occurs between the brains of the different castes or stages and but very little caste differentiation appears, although the optic apparatus shows a correlation between the degree of development of the compound eyes and the size of the optic lobes. The structure of the brain in termites resembles very closely that of ants, except that the mushroom bodies are much simpler and more primitive. The ocelli, present in the nymphs and adults of the sexual forms but absent in the worker or soldier, are simple, primitive, without lens or pigment, and lack the ocellar lobes of the ocellar nerves which occur in ants. The problematical frontal gland, found in all castes and situated on the postero-dorsal surface of the brain between the mushroom bodies, is composed of epithelial cells continuous with the hypodermis and innervated from the brain. It seems to be functional only in the true adult and soldiers. "The suggestion is made that the frontal gland may have arisen phylogenetically from the ancestral medial ocellus which is now lacking in the

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termites, and that the 'fontanel' nerve may be a vestige of the former median ocellar nerve."

Reflex "Bleeding."—McIndoo ('16, Annals Ent. Soc. Am., 9:201-222) finds that the "reflex bleeding" (the ejection of drops of liquid from the femoro-tibial articulations in certain coccinellid and meloid beetles) is a true reflex in *Epilachna borealis,* one of the *Coccinellidæ,* but that the liquid, instead of being blood, is a secretion from hypodermal glands and passes to the exterior through innumerable tubes opening near and in the articular membrane. Hypodermal glands are distributed widely over the integument of this species, groups of them occurring on the tarsi and around the femoro-tibial articulations, two at the proximal end of the tibia and two at the distal end of the femur. All four contain about 100 pores. The articular membrane contains about four hundred pores of still another kind. Fluid is emitted from these groups of pores in response to irritation. The discharge of the secretion is accomplished by muscular contraction in the femur whereby the blood is forced into a specially devised chamber containing the glands. The glands associated with the femoro-tibial articulation lack the reservoirs which characterize those glands distributed widely over the body. The secretion is bitter and disagreeable in odor. Its function is thought to be that of protection and it is suggested that possibly it aids in sex recognition and in distinguishing between different individuals.

Effect of Röntgen Rays.—Runner ('16, Journ. Agr. Research, 6:383-388) has experimented with the effect of Röntgen rays on the tobacco, or cigarette, beetle *(Lasioderma serricorne),* using a new form of Röntgen tube designed by Coolidge. Heavy dosages are demanded in the treatment of cigars or tobacco infested with this insect. Heavier exposures must be used for eggs near the hatching point than for those recently laid. Dosage equivalent to 150 milliampere minutes exposure with spark gap of 5.5 inches gave satisfactory results with eggs in tobacco placed 7.5 inches from the focal spot of the tube. Under this exposure, eggs in an advanced stage of development hatched but all observed specimens failed to reach the adult stage. Adults submitted to an exposure of 600 milliampere minutes, with spark gap of 5.5 inches, "giving

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an approximate voltage of 65,000," and distance from focal spot of tube being 7.5, apparently lived the usual length of time but the large number of eggs deposited after exposure were infertile. Larvæ, receiving the same treatment, showed decreased activity and development, remaining in a dormant condition for a considerable period, and all died before reaching the pupal stage.

Parasitized Larvæ of Army-worm.—Tower ('16, Journ. Agr. Research, 6:455-458), in a comparative study of the amount of food eaten by parasitized and nonparasitized larvæ of *Cirphis unipuncta,* has found that when attacked by an internal parasite *(Apanteles militaris),* the parasitized larvæ of the army-worm ate approximately half as much food as unparasitized larvæ during corresponding periods, indicating that the parasitism becomes directly beneficial in the generation attacked. Four newly molted fifth-stage specimens when parasitized ate respectively 16.21, 12.16, 11.97, and 14.50 square inches of corn foliage during the last two stages previous to the emergence of the parasites while the average of twenty nonparasitized larvæ during the same stages was 33.6 square inches. Five partially developed fourth-stage larvæ when parasitized ate respectively 20.63, 17.36, 21.24, 17.64, and 17.99 square inches, while twenty nonparasitized larvæ ate, on the average, 34.77 square inches during the same stages.

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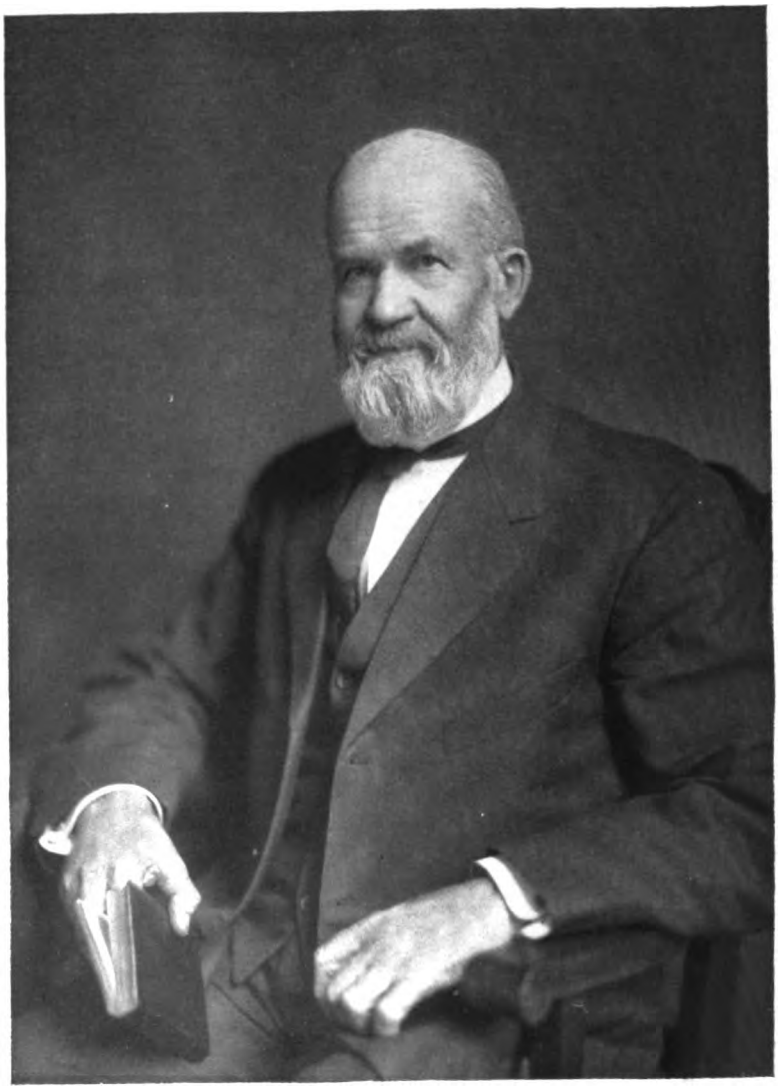

PLATE XL

THOMAS JONATHAN BURRILL

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THOMAS JONATHAN BURRILL

April 25, 1839 April 14, 1916

In the death of Professor Thomas Jonathan Burrill, the American Microscopical Society has lost one of its charter members whose activity in behalf of the Society may best be judged from his having twice served as its President and at another time as Secretary. A history of forty-four years of active service in one institution—the University of Illinois—where he watched the growth of teaching and investigation in natural science from the time when he was sole instructor in 'natural history and botany' to the time of his introduction of the laboratory method of instruction in his classes in that institution, and finally to the division of his work among four fully equipped independent departments,—all of them the direct outgrowth of his early enthusiasm for science; such is a history that is rarely recorded for any individual.

The first to discover a bacterial cause of disease in plants he opened a broad new field for investigation in bacteriology. His discovery of the cause of pear blight in 1880 assured for him a place as a leader in investigation but circumstances prevented his following this line of work. As one of his closest friends has expressed it "he loved people better than things, education better than science, and others better than himself; and he turned aside into what seemed to him the path of his duty, with no shadow of hesitation or appearance of regret, leaving it to others to note and remember his steady loyalty to his ideal." This turning aside was in response to the constant and growing demands of educational

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and administrative duties which prevented his devoting himself to extended investigations.

Professor Burrill retired from active service to the University in 1912. This same year he was made Professor Emeritus of Botany. His withdrawal from administrative duties gave him time even at his advanced age to begin again upon an active research program. Until within the week of his death he was busily engaged in attempting to induce nitrogen forming bacteria to grow on non-leguminous plants. His interest in modern problems of bacteriology was most fittingly recognized in his election to the Presidency of the American Society of Bacteriologists in December of the year preceding his death.

The development of biological equipment during a single life time is most vividly shown in the collection of microscopes and apparatus used by Professor Burrill and now on exhibition at the University of Illinois. One of his most intimate colleagues has promised an account dealing with his work and his apparatus for an early number of the Transactions.

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